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The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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Paul R. Miller

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Plant Industry Station, Beltsville, Maryland

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PHYSIOLOGIC RACES OF PIRICULARIA ORYZAE CAV.

Frances M. Latterell[1], Edgar C. Tullis[2], and Jack W. Collier[2]

Abstract

Fifteen physiologic races of Piricularia oryzae are characterized by the susceptibility reactions of 10 rice varieties. Isolates of similar pathogenicity were found from widely separated rice-growing areas of the world.

Physiologic races, defined as forms or strains of an organism that differ in the ability to attack the varieties of a single species of host plant, have not been demonstrated experimentally in Piricularia oryzae Cav., the causal fungus of the rice blast disease.

An epiphytotic of rice blast occurred in the new rice-growing areas of southern Florida in 1952 and 1953 and caused severe losses in plantings of Zenith, a variety that had been reported to be resistant to blast in Arkansas (13). This was the first positive evidence in the United States of the existence of physiologic races of P. oryzae.

Experimental evidence confirming the existence of at least two races of P. oryzae in the United States was reported in a preliminary abstract (6). Further studies have been undertaken to determine the extent of variation in pathogenic potentialities among isolates of P. oryzae from various geographical areas throughout the world. In the course of these studies, isolates of Piricularia from other hosts have been tested for pathogenicity to rice to determine if possible the sources of natural inoculum for epiphytotics of rice blast. The primary purpose of this paper is to provide information on the diversity of pathogenic forms which should be taken into consideration in breeding rice varieties for resistance to the rice blast fungus.

MATERIALS AND METHODS

Isolates of Piricularia spp. were obtained from leaf, culm, and panicle lesions from rice and other hosts (predominantly gramineous) from rice-growing areas in various parts of the world. The original isolates were transferred from tubes to 300-ml Erlenmeyer flasks of 2% rice polish agar poured to 1/2-inch depth. Flask cultures were incubated at room temperature (25° to 28° C) under continuous fluorescent light (200 fc) for 6 to 10 days, until spores were produced in sufficient numbers for use in inoculation tests.

The various isolates were tested for pathogenicity by spraying water suspensions of the spores onto the plants in the early tillering stage. Spores were harvested from the agar flask cultures by agitation with distilled water and glass beads. Spore concentrations in the resulting suspensions were determined before inoculation. In the cases of poorly sporulating cultures, the spore yield could usually be increased by discarding the first harvest, and re-incubating the unplugged flasks for about 48 hours.

The inoculated plants were placed in "dew chambers" (Mitchell and Cherry (7)) at 26° and 27° C for 16 hours, after which they were transferred to the greenhouse. Varietal reactions were recorded 5 to 7 days later; leaf specimens were mounted on cards under Scotch tape for future reference.

Eight varieties of rice representing different sources of germ plasm were used as standard test varieties in early screening tests. Subsequently, additions from the United States Department of Agriculture world rice collection were added as better differentiating varieties were found.

EXPERIMENTAL RESULTS

Cultures Obtained: More than 200 cultures of Piricularia were isolated, 165 of them were from rice from the United States and from 14 countries in Central and South America, Europe and Asia. The remainder were from other hosts as indicated: Stenotaphrum secundatum, Digitaria sanguinalis, Echinochloa crus-galli, Pennisetum purpureum, and Panicum purpurascens, all from Florida; Panicum miliaceum, Japan; Eleusine coracana, Saccharum officinarum,

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Table 1. Reactions[a] of rice varieties to two isolates of P. oryzae.

Source of isolate	Variety of rice			
	Zenith	Rexoro	LaCrosse	Caloro
Florida	+	+	-	-
Arkansas	-	-	+	+

[a] + susceptible, - resistant.

Setaria viridis, and Setaria lutescens, from Florida; Panicum repens, India; Kyllinga brevifolia, Florida; Zingiber officinale and Z. mioga, Japan.

Pathogenicity: Testing of isolates of Piricularia for pathogenicity to rice varieties was begun in October 1953, in which test an isolate from Florida was compared with one from Arkansas. The rice varieties Zenith, Rexoro, LaCrosse and Caloro were the test varieties in this series of inoculations. Responses are shown in Table 1. The reactions were consistent for each isolate and proved the existence of at least two physiologic races of P. oryzae.

Reaction Types: A range of six reaction types, from immune to highly susceptible, were characterized as follows:

Type 0 - immune, occasionally faint chlorotic flecks
Type 1 - pin-point brown lesions
Type 2 - small irregularly shaped brown lesions, 1 to 2 mm diameter
Type 3 - eyespots 3 to 4 mm in diameter with grey centers, intermixed with type 2 lesions
Type 4 - large greyish eyespots up to 30 mm in diameter
Type 5 - lesions at first as in type 4 but enlarging rapidly causing early death of the plants

Types 0 to 2 did not enlarge and did not sporulate. Type 3 lesions on exposure to dew did sporulate without, however, increasing in size and is regarded as a resistant type reaction. Types 4 and 5 are susceptible type reactions. The lesion types are shown in Figure 1.

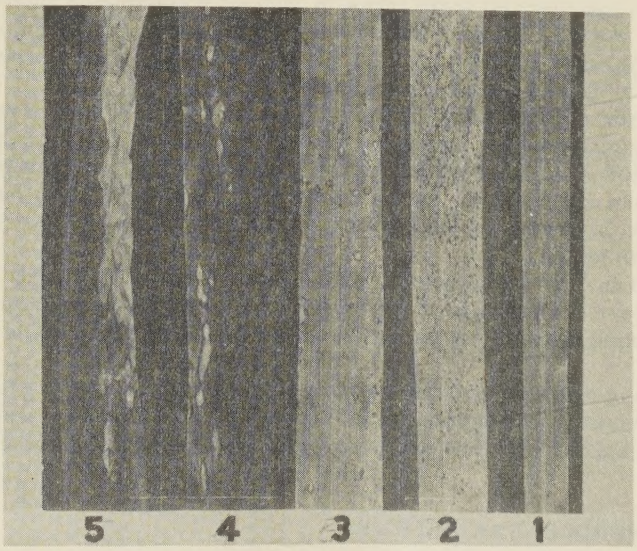

FIGURE 1. Lesion types. Numbers 1 to 3 are regarded as resistant reactions, and 4 and 5 as susceptible reactions.

Similar scales of reaction types were proposed independently by Hashioka (3) in Taiwan and by Padmanabhan and Ganguly (9) in India.

Selection of Test Varieties of Rice: In the initial stages of the screening of isolates eight varieties were used: Zenith, LaCrosse, Caloro, Rexoro, Blue Bonnet, Century 231, Nira, and C.I. 8970. All but C.I. 8970 were varieties grown on a commercial scale in the United States. C.I. 8970 was a chinese variety that proved to be a mixture which was purified and the two components identified by purple and straw auricles were both used. The varieties Zenith, LaCrosse, Caloro, and C.I. 8970(P) and C.I. 8970(S) were retained in the final test group and the varieties P.I. 180061 and P.I. 201902 from India, P.I. 231129 and P.I. 231128 from the Philippines, and C.I. 5309 from China were added to bring the number of test varieties to 10.

Table 2. Susceptibility of 10 varieties of rice to 15 races of Piricularia oryzae.

Race	Zenith	LaCrosse	Caloro	C.I. 8970(P)	C.I. 8970(S)	C.I. 5309	P.I. 180061	P.I. 201902	Wag-Wag	Raminad Str. 3
1	+[a]	-[b]	-	-	+	-	+	-	-	-
2	-	+	+	-	-	-	-	-	-	-
3	-	-	+	+	+	-	-	-	-	-
4	-	-	+	-	-	-	-	-	-	-
5	-	-	-	-	+	-	-	-	-	-
6	-	+	+	+	+	-	-	-	-	-
7	+	+	+	+	+	+	+	-	-	-
8	-	+	+	+	+	+	+	-	-	-
9	-	+	+	+	+	+	+	+	-	-
10	-	-	-	-	-	-	-	-	-	-
11	-	+	+	+	+	-	-	-	+	+
12	-	-	-	-	+	-	+	-	-	-
13	+	+	+	+	+	+	+	+	+	-
14	-	-	-	-	-	-	+	-	-	-
15	-	-	-	-	+	-	+	-	-	+

[a]reaction types 4 and 5.
[b]reaction types 0 to 3.

Table 3. Geographic distribution of 15 races of Piricularia oryzae.

Source	Number of isolates	Race 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Arkansas	2		X													
Louisiana	12		X		X			X								
Florida	13	X						X[a]								
Texas	4								X							
Mexico	9	X		X	X		X		X				X[a]			
Guatemala	1		X													
Nicaragua	14	X		X	X[a]		X[a]		X[a]				X			
Costa Rica	60	X		X	X	X	X[a]	X					X	X		
Venezuela	1	X														
Brazil	3		X													
Italy	13			X			X	X								
Greece	3			X			X									
Japan	3		X				X									
China	1				X											
Thailand	4		X													
Burma	1								X							
India	11			X			X		X		X[a]				X	X[a]
Philippines	10					X	X	X		X		X				

[a]cultural variants.

By the use of these 10 rice varieties, 15 races of Piricularia oryzae were identified among the 165 isolates from rice.

Identification of Races: The pattern of varietal susceptibility to the isolates is shown in Table 2.

In subsequent screening tests in which many rice varieties were inoculated under identical conditions the isolates were proved to be quite different from each other and it became apparent that they should properly be referred to as biotypes rather than races. Isolates of similar pathogenicity on the standard varieties were identified from various parts of the world, as shown in Table 3.

Some races, as numbers 1 and 12, were found in rather limited areas while others, as 2, 6, and 8, were found in widely separated areas.

There appeared to be little relationship between the number of isolates from an area and the biotypes identified. Among 60 isolates from Costa Rica there were 8 biotypes identified and from 12 isolates from India 6 biotypes were identified, and the same number from 9 isolates from Mexico.

Pathogenic Mutability: Cultural mutations were quite common on stock cultures of Piricularia oryzae. The variants were cultured and tested for pathogenicity on the standard varieties of rice. Eight of the variants proved to be pathologically different from the parent culture. These variants appeared in isolates from Florida, Mexico, Nicaragua, Costa Rica, and India.

Increase in virulence was found in some of the mutants and loss of virulence occurred in others. All mutations from the normal grey color to buff resulted in complete loss of pathogenicity to rice.

Cultural Characteristics and Spore Morphology: A wide range of colony types was encountered among the isolates, and a high degree of instability of cultural characteristics within individual isolates was common. Colony types exhibited many gradations from smooth, dark grey colonies of high sporulation capacity to cottony, white, or flat non-pigmented colonies of low sporulation. Similar gradations were found in the buff mutants mentioned before.

Among the cultures studied, only five maintained a relatively constant colony character. Single conidial transfers for several generations reduced variability in some isolates, but were ineffective in stabilizing others. No correlation was found between colony type and pathogenicity.

A comparatively wide variation in spore size was found among the isolates from rice, and from isolates from grasses. Variability was apparently influenced to a greater extent by cultural conditions (such as light) than by host.

Cross-infectivity of Rice and Grass Isolates: Selected isolates from rice were used to inoculate St. Augustine grass (Stenotaphrum secundatum), crab grass (Digitaria sanguinalis), para grass (Panicum purpurascens), sugar cane (Saccharum officinarum), and millet (Panicum miliaceum). Pin-point lesions were produced on some rice varieties by an isolate from St. Augustine grass, no infection was produced on rice by other grass isolates or isolates from sedge or ginger. Several isolates from rice produced typical eyespot lesions on St. Augustine grass and sugar cane.

DISCUSSION

The term "physiologic races" as used in this paper denote biotypes of the rice blast fungus that differ in their ability to attack rice varieties. They are characterized by their patterns of specialization in pathogenicity to a selected group of "differential" rice varieties.

The terms "physiologic races" and "physiologic forms" have been used in the literature dealing with the genus Piricularia to denote variability of the fungus in regard to cultural, physiological and biochemical characters and pathological variations on hosts other than rice. Tochinai and Shimamura (12) and Aoki (1) used one of these terms to refer to cultural variations among isolates, that is, amount of aerial hyphae, degree of sporulation, and coloration of submerged hyphae. Inoue (5) classified isolates into strains with respect to their ability to decompose cellulose. Strain differences in mode of spore germination and growth response to temperature were also reported (2, 12). Nisikado (8) concluded that "strains" of Piricularia on species of Setaria and Zingiber were distinct species. Venkatarayan (14) in 1947 without citing experimental evidence states that the blast fungus is "supposed to have a number of physiologic races, each capable of attacking only one or a few particular varieties, so that a variety that is susceptible to the disease in one place may be resistant to it in another place and hence the problem of breeding disease-resistant varieties against the disease is complicated." Hashioka (4) reported in 1952, however, that in extensive field trials at seven stations over a period of 5 years he did not obtain evidence of "races distinctly different in pathogenicity."

It appears, therefore, that the results of the present study provide the first definitive evidence of the existence of physiologic races of Piricularia oryzae, characterized on the basis of their ability to attack rice varieties differentially. As previously noted the "races" characterized herein should perhaps more correctly be called "race groups" for the races can, by increasing the number of rice varieties used as differentials, be further divided into biotypes.

Padwick (10), in his comprehensive review of the literature pertaining to the taxonomic status of species of Piricularia, has made evident the lack of a standard nomenclature for the species associated with leaf spots of grasses and rice. In general the rice blast fungus is referred to as P. oryzae, and other graminicolous species as P. grisea (Cooke) Sacc. Nisikado (8) emended the descriptions of these species and corrected errors in the original description of P. grisea (11), and placed several binomials in synonymy with P. grisea or P. oryzae. Nisikado also described as new species the strains causing leaf spot of Setaria spp. and Zingiber spp. The present authors, however, are of the opinion that the morphological similari-

ties of the graminicolous strains of Piricularia are so great that they should all be considered as P. grisea, the oldest accepted binomial, accompanied by an appropriate forma specialis designation, that is, P. grisea f. sp. oryzae for the rice blast fungus.

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CONTROL OF LIMA BEAN DOWNY MILDEW WITH PHENACRIDANE CHLORIDE AND CLOSELY RELATED COMPOUNDS

Bernard C. Smale and John W. Mitchell[1]

Summary

Phenacridane chloride (9-(p-n-hexyloxyphenyl)-10-methyl-acridinium chloride) and several close relatives were markedly effective as protectants of lima beans from downy mildew. Essentially complete protection from the mildew resulted from a 63-ppm spray of the fungicidally active compounds. The ED-50 values of the acridinium compounds indicated that under greenhouse conditions they were four to eight times as effective for downy mildew control as manganese ethylene bisdithiocarbamate.

INTRODUCTION

Although the antibacterial and antifungal properties of phenacridane chloride[2] against clinically important organisms have been reported (1, 2, 3), there are, as far as the authors know, no published reports on the effects of this compound on plant pathogens. The experiments reported were undertaken to learn whether this and related quaternary compounds might be of interest for plant disease control.

METHODS

Selection and Chemical Treatment of Plants: Fordhook lima beans grown in a greenhouse were selected for uniformity when the primary leaves were approximately 50% expanded and the secondary leaves were still tightly folded in the terminal bud. A glass chromatographic sprayer was used for spray application of the various aqueous preparations (to run off). For application of a chemical to the upper leaf surface only, a cotton swab saturated with a water mixture of a compound was used and a pad of paper toweling was held against the lower leaf surface to protect it from deposition of chemical.

Preparation of Chemical Mixtures: Aqueous solutions or finely dispersed suspensions of the quaternary ammonium compounds and a commercially used formulation of maneb (manganese ethylene bisdithiocarbamate) were prepared by dissolving or mixing the compounds with a small volume of 95% ethanol and adding distilled water. Sufficient ethanol was used to make a final alcoholic concentration of 1%. Lesser concentrations of the compounds were prepared by serial dilution, using 1% ethanol in distilled water as diluent.

Inoculation and Incubation: Inoculum of downy mildew of lima bean (Phytophthora phaseoli Thaxt.) was prepared within 1 hour of inoculation by washing zoosporangia from the surfaces of mildew-diseased lima bean hypocotyls and filtering the suspension through several layers of cheesecloth. The resulting zoosporangial suspension was examined microscopically for relative freedom from particles other than the pathogen and standardized photometrically to a 40% transmittance value in the wave length region of 400 to 465 mμ. About 1 hour after application of the chemical compounds, during which time the spray had dried on the plant surface, inoculum was applied with a glass sprayer mainly to the lower surfaces of primary leaves. Application of inoculum was limited so that no run-off occurred and redistribution of spray residue was minimized. Inoculation of the lower surfaces of primary leaves only was carried out with an artist's brush. Inoculations were confined by a pad of paper toweling held against the opposite surface.

Inoculated plants were kept at 65° to 75° F in a clear-plastic-covered enclosure for 2 days under conditions of high humidity. High humidity was produced with an automatic misting device operated for about 10 seconds every 10 minutes. This degree of humidification resulted in practically no run-off of water from primary leaves. Mechanical humidification was discontinued after 2 days, but plants were kept within the enclosure for an additional 12 days. Twelve hours of daily illumination was supplied during the 2-week period by fluorescent tubes which produced about a 500-foot-candle intensity. Under the conditions described, control plants

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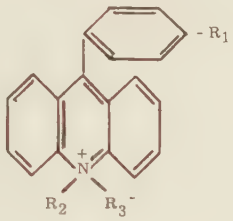
[2]This and related compounds supplied by Abbott Laboratories, North Chicago, Illinois.

Table 1. Effectiveness of phenacridane chloride and closely related compounds applied immediately before inoculation of lima bean leaves with the downy mildew pathogen.

Compound designation and name	Structural formula	Concentrations tested (ppm)	% disease control[a] 7 days	% disease control[a] 14 days	ED-50[b] End of 7 days (ppm)
"Parent compound"					
I. 9-(p-Methoxy-phenyl)-10-methyl-acridinium chloride	R_1 = OCH_3 R_2 = CH_3 R_3 = Cl	250 125 62 31	0 0 0 0	0 0 0 0	
II. 9-(p-n-Butoxy-phenyl)-10-methyl-acridinium chloride	R_1 = OC_4H_9-n R_2 = CH_3 R_3 = Cl	250 125 62 31	0 0 0 0	0 0 0 0	
III. 9-(p-n-Hexyloxy-phenyl)-10-methyl-acridinium chloride (Phenacridane chloride)	R_1 = OC_6H_{13}-n R_2 = CH_3 R_3 = Cl	250 125 62 31 8 2	100 99+ 99+ 87 74 15	100 97 89 44 20 0	5
IV. 9-(p-n-Octyloxy-phenyl)-10-methyl-acridinium chloride	R_1 = OC_8H_{17}-n R_2 = CH_3 R_3 = Cl	250 125 62 31 8 2	100 99 98 96 32 23	100 94 94 78 0 0	9
V. 9-(p-n-Decyloxy-phenyl)-10-methyl-acridinium chloride	R_1 = $OC_{10}H_{21}$-n R_2 = CH_3 R_3 = Cl	250 125 62 31 8	100 99 99 99 53	100 90 83 79 0	7
VI. 9-(p-n-Hexyloxy-phenyl)-10-ethyl-acridinium chloride	R_1 = OC_6H_{13}-n R_2 = C_2H_5 R_3 = Cl	250 125 62 31 8	100 100 98 82 46	100 88 78 71 0	10
VII. 9-(p-Methoxy-phenyl)-10-n-hexyl-acridinium chloride	R_1 = OCH_3 R_2 = C_6H_{13}-n R_3 = Cl	250 125 62 31	0 0 0 0	0 0 0 0	--
VIII. 9-(p-n-Hexyloxy-phenyl)-10-methyl-acridinium p-toluene-sulfonate	R_1 = OC_6H_{13}-n R_2 = CH_3 R_3 = p-toluene sulfonate	250 125 62 31 8 2	100 100 100 94 89 33	100 100 100 41 16 0	4
IX. 9-(p-n-Hexyloxy-phenyl)-10-methyl-acridinium picrate	R_1 = OC_6H_{13}-n R_2 = CH_3 R_3 = picrate	250 125 62 31 8 2	100 99 37 19 -- --	100 96 17 5 -- --	82
X. 9-(p-n-Hexyloxy-phenyl)-10-methyl-acridinium disalicylate	R_1 = OC_6H_{13}-n R_2 = CH_3 R_3 = disalicylate	250 125 62 31 8 2	100 100 95 85 68 26	100 99+ 80 44 25 0	8
XI. Manganese ethylene bisdithiocarbamate (maneb)		500 250 125 63 32	100 100 98 75 42	100 97 92 57 0	35

a % disease control data are average figures from at least five separate experiments involving a total of 50 plants per concentration.

b One-half the minimum amount required for complete disease control.

became severely and uniformly diseased within 5 days of inoculation and primary leaves, petioles, and first and second internodes were covered with signs of mildew.

RESULTS AND DISCUSSION

Fungicidal Properties of Phenacridane Chloride: Phenacridane chloride appears to be fungicidal rather than fungistatic in its effect on the downy mildew fungus. Zoosporangia exposed for 5 and 35 minutes to a concentration of 125 ppm of the compound failed to induce the disease when they were separated from the solution and used to inoculate plants.

The zoosporangia were intensely stained with the yellow fungicide as a result of either exposure. Attempts to remove the stain from the zoosporangia by repeatedly suspending and shaking them in distilled water neither visibly reduced the intensity of the color nor resulted in a return of pathogenicity.

Effectiveness of Phenacridane Chloride and Some Closely Related Compounds as Downy Mildew Protectants: Five compounds (IV, V, VI, VIII, and X, Table 1) closely related to phenacridane chloride (III) were found to be highly effective protectants of lima bean against downy mildew. These were approximately equal to phenacridane chloride in this respect. Since reduced water solubility, together with marked fungitoxicity, is often a desirable protectant characteristic, three compounds (VII, IX, and X) which have relatively low water solubility were of particular interest. Among the compounds tested, 9-(p-n-hexyloxyphenyl)-10-methylacridinium p-toluenesulfonate (VIII) seemed to be slightly more effective than the others.

The length of the hydrocarbon side chain associated with the phenyl ring was apparently involved in the degree of fungitoxicity exhibited by the different compounds (I through V), those with six carbon atoms or more being highly effective.

Therapeutic Inactivity: No disease control by any of the compounds was noted when they were applied only to the upper surfaces of leaves immediately before inoculation of the lower surfaces. This absence of therapeutic activity was observed with aqueous mixtures of phenacridane chloride (III) at concentrations of 1000 and 125 ppm and with 125 ppm preparations of other phenacridanes.

Phenacridane chloride was further studied with respect to its efficacy when applied at intervals following inoculation. Spray application of a concentration of 250 ppm to the lower primary leaf surfaces 2, 4, and 6 hours after inoculation resulted in complete or marked disease control, while essentially no reduction of disease severity was observed when application of the phenacridane chloride was delayed 8 hours. This result indicates that the acridinium compound was not absorbed by the plant in fungitoxic amounts or, if it was absorbed, it was inactivated before a fungitoxic effect could be produced.

Surfactant Properties of Some Phenacridane Compounds Related to Their Effectiveness as Protectants: Marked differences in the surfactant properties of compounds I through V were noted. Aqueous solutions (or mixtures) containing 250 ppm of the methoxy- and butoxy- phenacridanes foamed only slightly when shaken while the hexyloxy-, octyloxy-, and decyloxy- derivatives foamed readily. The octyloxy- derivative foamed about as much as did a 100-ppm aqueous mixture of Tween 20. The hexyloxy- derivative foamed about half as much as did the Tween. The role played by the surfactant property in control of the disease is not understood.

Phytotoxicity: The minimum spray concentration of phenacridane chloride that protected against downy mildew and the minimum concentration which caused phytotoxicity differed widely. Essentially complete mildew control was obtained with a 63-ppm spray while initial phytotoxicity occurred with a 2000 ppm spray. Other acridinium compounds were nonphytotoxic at the highest level tested (250 ppm).

This report is not intended as a recommendation for use of phenacridinium compounds in the production of edible crops.

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LIMITED SAPROPHYTIC SURVIVAL OF THE OAK WILT FUNGUS, CERATOCYSTIS FAGACEARUM (BRETZ) HUNT[1]

William H. Gillespie and Charles L. Wilson[2]

Summary

Results obtained from experiments designed to explore the natural survival of the oak-wilt fungus in duff, soil, leaves and on Hypoxylon stromata are described. Due to the limited nature of the experiments no specific conclusions are drawn. However, the organism survived for 70 days in the forest duff under rather unfavorable conditions and in competition with several fungi. If such survival occurs naturally, the areas involved could possibly serve as additional sources of inoculum for long-distance spread of the disease. If, in addition, the fungus is found to be capable not only of survival, but of sporulating and consequently multiplying in nature in media other than infected oak trees, the possible implications would be of prime importance to the States presently attempting control of the disease. Additional studies designed to answer these questions are vitally needed.

INTRODUCTION

Effective control of oak wilt depends to a degree upon clarification of the life history of the causal agent, Ceratocystis fagacearum (Bretz) Hunt. One of the more important unknowns is the period that the fungus can survive saprophytically outside of the affected tree in nature. Locations where the fungus might survive and multiply outside of the tree need investigation. Such investigations are pertinent to most control methods that are being recommended, since these controls are based on the assumption that the fungus in and on infected trees is the principal source of inoculum for overland spread.

This paper reports the results of preliminary experiments conducted from 1956 to 1959 in West Virginia and Arkansas. The work was initiated to explore certain aspects of the relationship of oak-wilt mats and a family of insects, the Nitidulidae, which are known to be vectors. One aspect of this relationship was pointed out by Dorsey and Leach (1) who stated, "the insects when disturbed on the mats often feigned death and dropped to the ground or flew away." These insects could carry the fungus to their hiding places -- such as in the duff or in hollow logs or stumps. If they did so, could the organism survive and serve as a source of inoculum for further spread?

Stambaugh, et al. (4) described experiments in which viable oak-wilt spores were recovered from the bodies of Nitidulid beetles. Using a modification of Jewell's (2) spermatization technique, Stambaugh and Fergus (5) demonstrated the longevity of ascospores to be at least 151 days on overwintering Nitidulid beetles in plastic cages buried in the forest floor. Controlled experiments by Merek and Fergus (3) show that conidia and ascospores survive best under cool, dry conditions. At humidities above 90%, which occur on the forest floor during the late fall and winter months, both conidia and ascospores succumbed within a few days, even at relatively low temperatures.

The present studies were undertaken to explore the possibility that C. fagacearum might survive under natural conditions of biotic competition on the forest floor. The possibility that the fungus might be present on Hypoxylon stromata, since Nitidulids have been observed on such structures, was also investigated.

EXPERIMENTS

During the early part of November 1956, live Nitidulid larvae and mycelial fragments from fresh oak-wilt mats were collected, placed separately in screen wire cages filled with randomly collected, non-sterilized oak leaves, and buried on the forest floor in Cooper's Rock State Forest, near Morgantown, West Virginia. Samples were placed in the duff layer, in the mineral soil,

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and in the decayed top of a stump about 4 inches below the surface of the rotten wood.

Representative larvae and mycelial fragments used as controls showed the presence of C. fagacearum in every instance when checked by the spermatization technique.

Owing to the preliminary nature of the experiments, only a few samples were buried. Some of these were removed at intervals of 8, 31, 62, and 70 days at which time all of the buried material was utilized. Since larvae were not found after the initial or 8-day recovery interval, subsequent tests were run by using the organic material that surrounded the point of larval placement. The samples were mixed with sterile distilled water, thoroughly macerated in a tissue grinder and used to spermatize six colonies of each compatibility type of C. fagacearum. Three additional colonies of each type were used as controls. The results of this experiment are presented in Table 1.

Table 1. Viability of C. fagacearum when buried in three locations on the forest floor.

Number of days in the field	Duff		Rotten stump top		Mineral soil	
	larvae	mat fragments	larvae	mat fragments	larvae	mat fragments
8	positive	positive	positive	positive	positive	positive
33	positive	not sampled[a]	positive	positive	positive	not sampled
62	not sampled	not sampled	negative	negative	not sampled	positive
70	not sampled	positive	not sampled	not sampled	not sampled	not sampled

[a] An insufficient amount of material prevented sampling each condition each time.

Fertilization was obtained with the Nitidulid larvae material under each of the three conditions at both 8 and 33 days. A single sample tested after 62 days was negative. It is significant that the fungus was recovered from organic material adjacent to where the larvae were placed. The mycelial fragments tested positively under at least one of the three conditions at every interval represented in the study. Samples buried in the duff layer survived 70 days. In addition to C. fagacearum, each plate consistently produced Trichoderma sp., Graphium sp., and Gliocladium sp. Bacteria and nematodes were often noted. The air temperature during this experiment varied from a high of 60° F to a low of -9° F, with a maximum daily difference of 50° being recorded. Temperatures ranged above freezing during at least 58 of the 71 days of the study.

Throughout the course of this work several related accessory experiments were conducted. They were summarized as follows:

1) Duff from beneath the crown of a healthy oak tree was collected, placed in Petri dishes, sterilized in an autoclave and inoculated with C. fagacearum. After 2 weeks, the characteristic felt-like cushions containing perithecial initials were formed. Perithecia developed about four days after spermatization with the opposite compatibility group. Later duplications of this procedure produced varied results, but two out of seven replicates produced fertile perithecia.

2) Oak leaves from underneath naturally infected oaks of the current year were collected in November. When this material was macerated and used to spermatize oak-wilt mats, the results were negative in 15 attempts from 15 different trees. However, positive results were obtained from leaves lying under mat-bearing bark fragments which had been on the ground for about 3 months.

3) Twenty-one agar cultures of C. fagacearum were removed from Petri dishes and buried in the duff layer on April 4, 1957. When recovered on July 26, these gave negative results when tested by the spermatization technique. Fifteen similar cultures were buried on August 12 and recovered and tested August 15, August 30, September 7, and September 16. Positive results were obtained only from a single sample collected August 15.

4) Duff, soil, and leaves were collected from a mixed stand of red and black oaks and placed in 8-inch clay pots, 2 inches deep. The pots were then buried with the rim parallel to the soil surface and the material was thoroughly wetted with a spore suspension of C. fagacearum grown in a liquid culture of 20% V-8 juice. Samples were collected in sterile, distilled water and used to spermatize cultures of the oak-wilt fungus. Positive results were obtained after 14 and 19 days, but negative results were obtained after 53 days when a rather extensive test was made. Unfortunately, circumstances prevented testing during the interval between the 19th and 53rd day.

5) Hypoxylon puntulatum (Berk. & Rav.) Cke. commonly invades trees affected by the oak-wilt fungus and forms stromata in the bark of diseased trees, whereas oak-wilt mats are typically found at the juncture of the bark and wood. Since Nitidulid beetles have been observed on these stromata, the possibility that C. fagacearum might be present on them was also investigated. On November 27 bark was collected from 20 blackjack oaks that had wilted during the spring and summer of 1958 in Arkansas. The surfaces of over 50 stromata of H. puntulatum were scraped and the scrapings were added to sterile water. This material was then used to spermatize both A and B cultures of C. fagacearum. Scrapings from two stromata collected on separate trees caused perithecia to be produced in the cultures. Over 60 stromata from 20 trees were collected April 8, 1959 and all yielded negative results.

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STIMULATION OF WALNUT BY SCHRADAN (OMPA) IS NOT THE RESULT OF ROOT-LESION NEMATODE CONTROL

B. F. Lownsbery[1]

Summary

Foliar sprays of schradan (octamethyl pyrophosphoramide) did not reduce the number of root-lesion nematodes (Pratylenchus vulnus Allen & Jensen) in the roots of nematode-infected California black walnut (Juglans hindsii) seedlings. This greenhouse test provided no evidence that nematode control is the mechanism for a suspected stimulation of walnuts by schradan.

INTRODUCTION

At present schradan is the insecticide most satisfactory (3) for control of the walnut aphid (Chromaphis juglandicola (Kltb.)). In field tests walnut growth improvement resulting from schradan sprays was so marked that a stimulating effect, beyond the control of aphids, was suspected (4). Because trees showing this striking response were infected with the root-lesion nematode Pratylenchus vulnus (1), the effect of this insecticide on populations of P. vulnus in walnut roots was studied.

MATERIALS AND METHODS

A quantity of soil from a San Joaquin County walnut orchard, infested with P. vulnus, was thoroughly mixed, and divided into two portions. One of these portions was treated with methyl bromide at the rate of 100 cc per 35 gallons of soil. The other portion was left untreated. Forty very young walnut seedlings were planted in 6-inch pots of the untreated soil. Forty other young seedlings were planted in the methyl bromide fumigated soil. P. vulnus was recovered from this untreated soil at the rate 303±75 nematodes per pot. No P. vulnus was recovered from the fumigated soil.

Table 1. Final numbers[a] of Pratylenchus vulnus per gram of root, and per pot (soil and roots), resulting from different schradan treatments of walnut seedlings infected with this nematode.

Number of P. vulnus:	Grams schradan per walnut seedling			
	0 (control)	0.002	0.02	0.2
Per g of root	102±16	103±16	66±10	107±33
Per pot[b]	12765±1203	16290±1652	14010±2160	10950±3114

[a]Means of 10 replicates, with their standard errors; none differ significantly from the controls.
[b]Initially 303±75.

Table 2. Final heights[a], in mm, of nematode infected and uninfected walnut seedlings treated with schradan.

	Grams schradan per walnut seedling			
	0 (control)	0.002	0.02	0.2
Untreated soil	181±11	195±10	210±9[b]	193±14
Methyl bromide fumigated soil	198±7	221±6[b]	209±6	190±12

[a]Means of 10 replicates with their standard errors.
[b]Differ significantly from their controls ($P<0.05;>0.01$).

When the seedlings had grown in these soils 3 weeks, in a greenhouse kept at approximately 26° C, they were sprayed with schradan at one-half the dosage rates shown in Tables 1 and 2. Another spray treatment at the same rates was given 1 month later. Ten ml of water per pot was used in the first application, 30 ml per pot in the second, when walnut seedlings were

[1]Technical assistance was given by J. T. Mitchell.

larger. Spray applications were made with a plastic atomizer of the kind sold with window cleaning solutions.

Two months after the first spray application seedling heights were measured, roots were weighed, and numbers of P. vulnus in roots and soil were determined. Nematodes were extracted from the soil by a variation of the Baermann method (2), from roots by a modification of Taylor and Loegering's (5) procedure. The entire root was blendorized in 100 ml water. Then, without any screening, the number of P. vulnus in an aliquot was counted. Total numbers of P. vulnus in the roots and in the soil were obtained by multiplying by appropriate factors.

RESULTS

Schradan is applied in walnut orchards at the rate of 1 pound per acre, or approximately 0.01 g per square foot. The test reported here included rates both above and below this level (Tables 1 and 2). At these rates, the schradan sprays produced no statistically significant effects on the final population levels of P. vulnus (Table 1). There is a suggestion (Table 2) that schradan at the two lowest dosage rates may have stimulated walnut seedling growth. The experiment provides no support for the hypothesis that schradan stimulates growth because of nematode control.

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PHYTOPHTHORA ROT OF WATERMELONS ON THE MARKET

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Summary

Shipments of watermelons from Arizona, California, and Mexico frequently have heavy loss during transit and marketing. Cultures from diseased melons show that species of Phytophthora cause most of the decay. Wounds are not necessary for infection to occur. Spread of the fungus from one melon to another in the load accounts for much of the decay in transit. P. capsici is one of the most important pathogens.

In recent years many shipments of watermelons from Arizona, California, and Mexico have had severe loss from decay during transit and marketing. This was first brought to our attention in 1953 by J. R. Hailey of the Western Weighing and Inspection Bureau at Denver, Colorado. Inspectors of this agency located at Denver, Spokane, Seattle, Tacoma, Portland, and Salt Lake City, where most of these melons are marketed, found excessive amounts of decay in many carlot shipments. Since watermelons from the above-mentioned regions seldom reached the Chicago market the writers agreed to cooperate with the Western Weighing and Inspection Bureau to determine the cause of this trouble by diagnosing the diseases of typical specimens sent to our laboratory.

At intervals during 1953 and 1954, 26 diseased specimens of watermelon were received from different cars inspected on the western markets. Cultures were made of the organisms within the diseased tissues. In practically every instance the isolates proved to be species of Phytophthora. The seriousness of this disease is well illustrated by a car of Peacock melons shipped from El Centro, California to Spokane, Washington in July 1953. A total of 347 melons affected with Phytophthora rot was removed from this car. A conspicuous white to gray mycelium was evident on a great number of melons and in many instances the mycelium had spread from one melon to another, creating nests of decaying melons (Fig. 1). The location of the decaying melons showed that many contact infections had occurred during transit.

FIGURE 1. Phytophthora spreading from one melon to another in the load.

In 1958 and 1959 over 200 cars of Mexican watermelons were inspected at the Chicago market on arrival and many were found to have a high percentage of Phytophthora rot. In some cars more than 300 melons were affected by this disease.

There is considerable variation in the amount of Phytophthora rot in different areas and in different years. In 1953 to 1956 the Arizona and California shipments inspected showed from 0 to 50% of the cars with some Phytophthora rot. In 1954, 1234 cars of Arizona and California

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watermelons were inspected on the western markets and 26% of them had Phytophthora rot. This decay was present in 14% of the shipments from central California, in 34% of those from the Imperial Valley, and in 32% of the Arizona shipments.

In laboratory inoculation tests, the Peacock, Klondike, Black Diamond, Charleston Gray, and Cuban Queen varieties of watermelon were found about equally susceptible to the disease. Each of these varieties developed decay rapidly following wound inoculations with isolates made from Arizona, California, and Mexican melons. When held at 70° F most melons inoculated at slight wounds in the rind developed greenish-brown lesions 2 1/2 to 4 inches in diameter in 5 days. Melons treated in a similar manner and held at 54° for 5 days developed lesions varying from 1/2 to 2 inches in diameter. Many inoculation tests indicated that no decay would occur in melons held at 40°.

The Peacock and Klondike varieties commonly shipped in the west were found to be very susceptible to Phytophthora infection even without any visible injury to the rind. Contact inoculation tests with pure cultures of Phytophthora growing on agar and also with small pieces of decaying watermelon tissue placed on clean uninjured areas of melon rind showed that visible infections occur within 2 days at 75° F. Such lesions were about 3 inches in diameter by the fourth day.

A Peacock melon with a large Phytophthora lesion (such as shown in Figure 2) was placed against a similar melon that showed no visible injury at the point of contact with the decaying specimen and held for 2 1/2 days at 75° F. During this time a lesion 3 inches in diameter developed on the healthy melon at the point of contact with the diseased one. Three days later this lesion was 7 inches in diameter -- indicating the rapidity with which Phytophthora infections may spread from one melon to another.

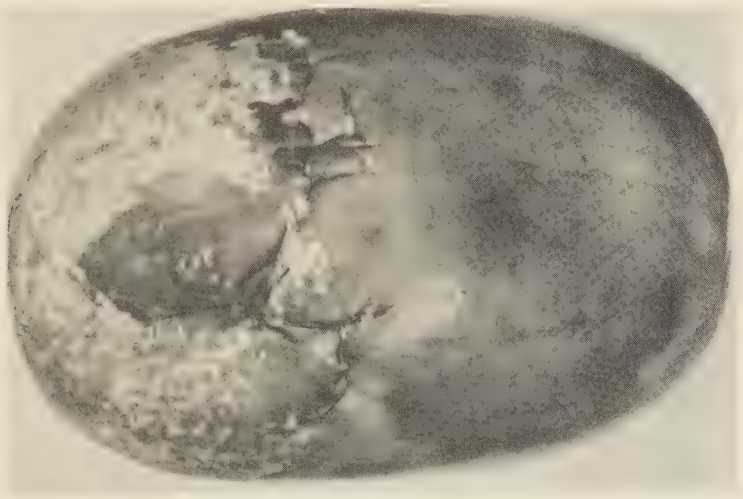

FIGURE 2. Typical Phytophthora rot of watermelon on the market.

In seasons favorable for Phytophthora infections, undoubtedly many melons bear inconspicuous lesions that may be unobserved at loading time. When these infections develop the fungus may spread by contact to other melons in the load. Without doubt such infections account for a large amount of the Phytophthora decay that occurs during transit and marketing of watermelons.

There are several reports of Phytophthora spp. affecting watermelons; the most important appear to be P. cactorum in Arizona (1), P. parasitica in Texas (4), P. citrophthora in California (3), and P. capsici in Japan (2). The last species also sometimes causes serious decay in honeydew melons in California (5) and in Winter Queen melons from Colorado (6).

Studies on the growth characteristics and spore measurements of a large number of watermelon isolates indicate that one or more of the above species account for the decay found in the shipments of melons from Arizona, California, and Mexico. Cultures made from many watermelons studied in this investigation showed that P. capsici was one of the most important pathogens.

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NEGATIVE TRANSMISSION OF AN APPLE VIRUS THROUGH SEEDS

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When it was discovered that the dwarf fruit virus (1) gave symptoms typical of the apple chlorotic leaf spot syndrome on the indicator clone R12740-7A (1, 2) an opportunity was presented to determine the extent of seed transmission.

Fruit was collected from a dwarf fruit inoculated Hyslop tree that had shown symptoms for over 10 years. All the fruit on the tree showed the typical dwarf fruit symptoms, being oblate and deeply scalloped. The tree, as in other dwarf fruit infected Hyslop trees, showed prominent stem pitting. It had also transmitted a necrotic leaf spot and stem pitting to some, but not all, Hopi and Zumi crabapple seedlings.

The seeds were vernalized, sprouted and planted in flats in the greenhouse during January and February 1960. Meanwhile, the indicators were prepared by grafting two buds of the clone R12740-7A to potted year-old domestic seedling apple trees by chip buds placed 10 to 12 inches above the soil line. When the indicator buds sprouted, tissue from five to ten of the Hyslop seedlings was grafted to each indicator tree.

The inoculations were conducted through February to May. About half the indexings were made by grafting the entire top of a 2- to 3-leaf seedling into a T-shaped cut on the indicator tree trunk. The Hyslop seedlings were pulled from the soil and the stem cut diagonally above the roots to expose meristematic tissue. After the cut stem of the seedling was inserted and the T-shaped cut taped to exclude air, the entire tree was covered with a polyethylene bag to prevent drying. Remarkable success was attained and in most cases the grafted seedlings became permanently joined. When the Hyslop seedlings reached larger size and became hardened only bud tissue was used for inoculation.

At first the Hyslop seedlings were selected at random. Later a special effort was made to include those seedlings showing stunting or virus-like leaf patterns. These abnormalities proved to result from genetic or physiological causes.

In all, 116 seedlings were indexed on a total of 18 trees. All the R12740-7A indicator shoots remained normal after observations extending from 6 months for the early inoculations to 6 weeks for the latest.

Comparative inoculations with bud tissue from the parent Hyslop tree run concurrently gave positive symptoms in every case. For the most part, these symptoms consist of epinasty of the terminal shoot, cessation of growth at the onset of symptoms, variable amounts of leaf mottling and in some cases necrosis of the shoot tip.

Approximately 600 domestic seedling apple trees were used to propagate R12740-7A buds during the 1959 and 1960 greenhouse seasons. Most of these buds grew normally. In the several cases of bud failure, the seedling trees were indexed and found normal. Thus, no virus reaction has been induced from some 716 seedlings, 116 of which were produced from a known diseased tree.

SUMMARY AND DISCUSSION

Evidence of seed transmission of the virus or viruses responsible for the chlorotic leaf spot syndrome was negative. The transmission of the stem pitting and dwarf fruit factors present in the dwarf fruit selection used in this test awaits further testing.

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NEW OR UNUSUAL DISEASES OF CEREAL CROPS IN VIRGINIA

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Crazy top of corn, caused by Sclerospora macrospora, was observed in Nansemond County in 1958 where it was found in two fields which were several miles apart. In one field, the disease occurred in only two outside rows for a distance of 300 feet. Apparently, at the critical time for infection, only this portion of the field was flooded. In the second field, by contrast, 100% of the corn plants observed were infected. This field, however, had been seeded with a mixture of corn and tall sorghum and was being cut for silage. No evidence of infected sorghum could be found. The field was judged to be about 10 acres and no normal ears of corn could be located, but since the crop was being used for silage, one could not determine the loss of food value caused by the disease. This appears to be the only record of crazy top in Virginia.

Soil-borne mosaic of oats was observed in yield tests at Charlotte C. H. in 1959. Varieties whose response to soil-borne mosaic had been determined in previous tests in North Carolina gave characteristic responses for soil-borne mosaic. There is no previous record of oat soil-borne mosaic in Virginia.

Halo blight, caused by Pseudomonas coronofaciens, was widespread and quite destructive in many areas in Virginia in 1960. Such destruction has not been observed before, and it is believed that the development of halo blight was related to the heavy snow cover which was also unusual for Virginia. Snowstorms beginning in mid-February and occurring at almost weekly intervals until mid-March resulted in snowfall exceeding 70 inches in some mountainous counties for that period. Heavy snow cover was statewide for 4 to 6 weeks. Several complaints of oat stands dying out were received in early April, and halo blight was found to be responsible for the trouble. The disease killed large areas in fields of oats in eastern Virginia where Arlington was the variety most severely damaged in commercial fields, but Victorgrain and Forkedeer were blighted also. In our experimental nurseries, severe damage occurred at Orange and Warsaw, with lesser damage occurring at Blacksburg, Charlotte C. H., Emory, and Petersburg. Where the severity of the disease was estimated, Dubois (C. I. 6572), Midsouth (C. I. 6977), and Victorgrain 48-93 (C. I. 7125) were resistant (trace to 3% foliage damage); Earlygrain[1], Fulgrain (C. I. 5336), and Woodgrain[1], were intermediate (5 to 12% foliage damage); and Arlington (C. I. 4657), Atlantic (C. I. 4599), Bronco (C. I. 6571), Forkedeer (C. I. 3170), Fulwood (C. I. 6584), Lee (C. I. 2042), Moregrain (C. I. 7229), and Suregrain (C. I. 7155) were susceptible (15 to 45% foliage damage).

Halo blight lesions on oat leaves are of common occurrence in Virginia but heretofore we had never seen any killing nor any record of killing of winter oats attributable to Pseudomonas coronofaciens. This bacterium, nevertheless, was isolated from the crowns of the chlorotic oat plants in the jointing stage, and it was observed that similarly affected plants died. Invasion of the crowns apparently was facilitated by infection of short, mid-winter produced leaves during the snow cover or shortly thereafter. Once the bacterium infected these short crown-leaves, it spread down the midrib into and throughout the crowns causing the plants to become chlorotic, stunted, and later to die. Killing of all plants in areas as large as 80 square feet was observed but usually only scattered patches of plants died. Although seedling plants of spring oats are reported to be easily killed by halo blight, there is no suggestion by either Manns (3), Elliott (1, 2), or others that fall sown oats may be killed in the spring by this disease.

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[1]Variety produced by T. W. Wood and Son, Richmond, Virginia.

BLIGHT OF PILEA MUSCOSA CAUSED BY COLLETOTRICHUM CAPSICI

H. K. Saksena[1]

Abstract

A hitherto unrecognized blight disease of Pilea muscosa, a perennial ornamental, is reported as caused by Colletotrichum capsici. Cross inoculation studies have shown this blight fungus to attack Capsicum annuum and Tagetes erecta, and C. capsici from these two hosts to attack P. muscosa.

Pilea muscosa Lindl. (Urticaceae) is a perennial, weedy herb of tropical and sub-tropical regions. Owing to its compact, fern-like sprays and gracefully curving fronds of small, shining leaves, it is a popular ornamental in conservatories and gardens and as a house plant. A blight disease of P. muscosa was first observed in July 1955 in plants growing in pots at the residence of the author in Kanpur, India. The disease was observed in subsequent years in several other gardens in the city. The damage was particularly serious in edgings and borders of P. muscosa where gaps were caused in the stand due to the death of the affected plants.

In nature the disease first shows up on the young leaves and shoots and then progresses downward to the branches and the main stem. As a first symptom, the small, ovate leaves become water-soaked, soft and glassy. Later they shrivel and turn black. After these foliage effects become general, the plant presents a parched appearance. Defoliation soon occurs and the top surface of soil underneath is covered by fallen leaves. After the leaves are shed, brownish water-soaked lesions, up to 2 inches long, become visible girdling the soft, succulent stem. Necrosis followed by collapse of the inner cells causes shrinkage of the shoots, which topple over and rot away. Black, dot-like acervuli appear in great numbers on the dead branches of infected plants. The conidia from these continue to spread the secondary infection.

Continuous observations on this hitherto unrecorded disease of P. muscosa indicate that high temperatures and high humidity favor the rotting. Disease symptoms manifest themselves quickly and blight is severe if the inoculated plants are kept in moist chambers. In nature, too, the disease is more severe in the warm and wet monsoon months than at other times.

Isolations made in October 1957 from diseased parts of P. muscosa yielded a Colletotrichum. The morphology and pathogenicity of this organism was studied by methods reported earlier[2]. The hyphae in culture measured 4-9.3μ in width. The average size range of other structures produced in culture and on host plant was: acervuli, 84-176 μ; setae, 50-198 x 2.5 -6μ; conidia, 18.3-27.6 x 2.3-3.5 μ; and conidiophores, 15.2-28.4 x 2.5-4.6 μ. The measurements of conidia of this fungus fall within the range given for C. capsici (Syd.) Butl. & Bisby.

The pathogenicity of this fungus was established by reproducing the disease on artificially inoculated P. muscosa plants. In cross inoculation tests the blight organism of P. muscosa caused mild dieback of fruits of Capsicum annuum and leaf and stem blight of Tagetes erecta. Isolates of C. capsici from C. annuum and T. erecta[2] successfully attacked P. muscosa. These studies further confirm the identity of C. capsici as the cause of blight of P. muscosa. C. capsici is known to parasitize a great variety of plants; a new host, P. muscosa, is now added to the list.

T. erecta is one of the most popular ornamentals that grow well in India during summer months. It is susceptible to C. capsici[2]. P. muscosa, being a perennial host, is well suited to harbor this pathogen all year round. To minimize the chances of spread of C. capsici to T. erecta, T. erecta should not be planted close to where P. muscosa is growing.

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[2]Saksena, H. K., and B. B. Singh. 1959. Blight of marigold, Tagetes erecta, caused by Colletotrichum capsici (Syd.) Butl. & Bisby. Plant Disease Reptr. 43: 670-673.

CONTROL OF POTATO SEED-PIECE DECAY[1]

Roland F. Line and Carl J. Eide[2]

Summary

In three consecutive years cut potato seed pieces produced lower yields if stored several days after they were inoculated and dipped in chemical seed-piece protectants. Even when the seed was stored, inoculation with Fusarium spp., soft-rot bacteria, or a mixture of both, often did not increase decay, which may occur without inoculation. Seed-piece decay before planting reduced stands, and stunted plants were correlated with lowered yields. Maneb was generally superior to a mixture of streptomycin and oxytetracycline, and equal to a mixture of all three as a protectant against decay.

INTRODUCTION

Chemical protectants to control potato seed-piece decay are recommended in some places and not in others (6). In Minnesota they have not been recommended because they have failed to improve stands or yields in a majority of field tests. It is not known whether the lack of benefit in Minnesota resulted from a relative absence of decay organisms in the soil or was due to the ineffectiveness of the protectants tried. New chemicals, especially antibiotics, have recently been introduced and tested as protectants at several places (1, 2, 3, 4, 7, 8). Two of these materials were tested during 1957, 1958, and 1959 on seed pieces inoculated with common decay-producing organisms. The effect of storing the cut and treated seed before planting was also determined.

MATERIALS AND METHODS

In 1957 freshly cut, uniform seed pieces of the Irish Cobbler variety were placed on screens and sprayed uniformly with inoculum of two isolates of Erwinia atroseptica (Van Hall) Jennison, two isolates of Erwinia carotovora (L. R. Jones) Holland, and four of Fusarium spp. from decayed tubers. Spore and bacterial suspensions were washed with distilled water from 5-day-old cultures on potato-dextrose agar in Petri dishes. The suspensions were prepared just before use and maintained at 36° to 40° F to reduce germination until they were sprayed on the seed pieces. Four lots of seed pieces were sprayed with the following: 1) a mixture of four isolates of Fusarium spp.; 2) a mixture of four isolates of Erwinia spp.; 3) a mixture of 1) and 2); and 4) a water check.

After drying 5 minutes, portions of each of the above were dipped for 1 minute into one of the following: 1) Agrimycin[3], 200 ppm; 2) maneb (manganese ethylene bisdithiocarbamate), 1 pound to 10 gallons of water; 3) a combination of 1) and 2); and 4) a water check.

Four kinds of inoculum (including the check) and four protectant chemicals (including the check) resulted in a total of 16 treatment combinations, and are referred to as such in the rest of the paper.

The treated seed pieces were air dried, put into cotton bags and the bags stored together in metal containers at 70° to 80° F. After 5 days a second lot was treated in the same way as the first, and both were planted on the same day that the second lot was treated.

The field test was designed as a split-split plot randomized block, with protectants randomized within kinds of inoculum and storage periods randomized within protectants. Each individual combination of protectant, inoculum, and storage period comprised six replicates of 12 seed pieces each.

Data were recorded and analyzed on tuber damage before planting, stand, plant height, and yield. Means were compared by the Student-Newman-Keul multiple range test as outlined by Federer (5).

The materials and methods in 1958 and 1959 were the same as those in 1957, except for the following changes: In 1958 the bacterial inoculum consisted of soft-rotted tissue washed

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[2]Research Fellow and Professor, respectively, Department of Plant Pathology and Botany, University of Minnesota, St. Paul.

[3]Agrimycin is a trade name for a mixture containing 15% streptomycin and 1.5% oxytetracycline plus inert ingredients.

Table 1. Yields from potato seed pieces dipped in chemical protectants after inoculation. Rosemount Experiment Station, 1957, 1958, and 1959.

Inoculum	1957		1958		1959	
Seed planted immediately after inoculation and dipping						
Fusarium spp.	maneb	28.2[a,b]	maneb	15.2	Agrimycin	13.3
	Agrimycin	28.0	man. + Agri.	15.0	none	12.6
	none	26.8	Agrimycin	13.5	maneb	12.3
	man. + Agri.	26.0	none	12.1	man. + Agri.	10.8
Bacteria	Agrimycin	26.5	man. + Agri.	14.9	maneb	17.0
	man. + Agri.	24.9	none	14.5	none	15.1
	none	24.6	maneb	13.7	Agrimycin	15.1
	maneb	23.4	Agrimycin	12.1	man. + Agri.	13.9
Fusarium spp. and Bacteria	maneb	26.5	man. + Agri.	14.5	none	15.8
	man. + Agri.	26.0	maneb	14.2	man. + Agri.	15.0
	Agrimycin	24.9	Agrimycin	14.2	maneb	14.6
	none	24.0	none	13.8	Agrimycin	12.9
None	Agrimycin	27.3	maneb	13.7	Agrimycin	15.9
	maneb	26.8	man. + Agri.	13.7	none	14.6
	man. + Agri.	24.6	none	12.8	maneb	14.0
	none	24.6	Agrimycin	12.6	man. + Agri.	12.9
Seed planted 3 to 5 days after incubation and dipping						
Fusarium spp.	maneb	23.5	man. + Agri.	13.0	maneb	13.7
	man. + Agri.	21.2	maneb	12.7	none	13.7
	Agrimycin	20.9	Agrimycin	8.8	man. + Agri.	12.6
	none	20.4	none	0.0	Agrimycin	12.0
Bacteria	Agrimycin	24.2	maneb	12.3	maneb	19.3
	man. + Agri.	22.2	man. + Agri.	10.1	man. + Agri.	15.9
	none	14.8	Agrimycin	7.8	none	5.8
	maneb	10.3	none	0.0	Agrimycin	0.0
Fusarium spp. and Bacteria	man. + Agri.	22.1	maneb	13.8	none	17.3
	Agrimycin	20.7	Agrimycin	1.6	man. + Agri.	16.4
	maneb	18.0	man. + Agri.	0.5	maneb	15.4
	none	14.6	none	0.0	Agrimycin	13.6
None	maneb	24.4	man. + Agri.	13.3	none	14.3
	none	23.7	maneb	7.2	maneb	13.5
	man. + Agri.	22.8	Agrimycin	2.1	man. + Agri.	12.8
	Agrimycin	15.8	none	0.0	Agrimycin	0.0

[a]Figures are means in pounds from six 12-hill rows.
[b]Figures bracketed are not significantly different statistically at 5% level, multiple range test.

from infected tubers with distilled water, and the treated seed pieces were stored for 3 days in cloth sacks each within a polyethylene bag, instead of in a metal container. The polyethylene bags prevented inoculum from soaking from one cloth sack to another. In 1959 the materials and methods were the same as those in 1958 except that the bacterial inoculum consisted of four unidentified isolates of soft-rot-producing bacteria.

RESULTS AND CONCLUSIONS

In each year there was a high correlation between yield data and those of tuber damage before planting, stand, and plant height. Because of the apparent association between these phenomena, only the means for yields are presented in this paper (Table 1). For simplicity, the data in this table are grouped by years and inocula and statistically significant differences between protectants in these groups are indicated. Other differences discussed in the paper are

all statistically significant, although this is not indicated in the table.

Inoculation with seed-rotting organisms had little effect on seed planted immediately, as shown by the data in Table 2, which are taken from Table 1.

Table 2. Effect on yields of inoculating cut potato seed pieces. No chemical protectant applied subsequently.

Inoculum	1957 not stored	1957 stored	1958 not stored	1958 stored	1959 not stored	1959 stored
Fusarium	26.8[a]	*20.4[b]	12.1	*0.0	12.6	13.7
None	24.6	23.7	12.8	*0.0	14.6	14.3
Fusarium + Bacteria	24.1	*14.6	13.8	*0.0	15.8	17.3
Bacteria	24.6	*14.8	14.5	*0.0	15.1	*5.8

[a]Figures are pounds per row of 12 hills.

[b]Figures bracketed are not significantly different; asterisks indicate that the yields are significantly lower than those from the corresponding non-stored lot. All differences calculated at 5%.

Table 3. The relative effectiveness of chemical protectants on yields from potato seed pieces inoculated and stored 3 to 5 days before planting[a].

A. Maneb superior to:

Inoculum	maneb	Agrimycin	maneb + Agrimycin	check	total
Fusarium spp.	--	2	1	2	5
Bacteria	--	2	1	2	5
Fusarium + Bact.	--	1	1	2	4
None	--	3	0	1	4
Total	--	8	3	7	18

B. Agrimycin superior to:

Inoculum	maneb	Agrimycin	maneb + Agrimycin	check	total
Fusarium spp.	0	--	0	1	1
Bacteria	1	--	1	2	4
Fusarium + Bact.	1	--	0	1	2
None	0	--	0	0	0
Total	2	--	1	4	7

C. Maneb plus Agrimycin superior to:

Inoculum	maneb	Agrimycin	maneb + Agrimycin	check	total
Fusarium spp.	0	1	--	1	2
Bacteria	1	2	--	3	6
Fusarium + Bact.	1	0	--	1	2
None	1	3	--	1	5
Total	3	6	--	6	15

D. No protectant superior to:

Inoculum	maneb	Agrimycin	maneb + Agrimycin	check	total
Fusarium spp.	0	0	0	--	0
Bacteria	1	1	0	--	2
Fusarium + Bact.	0	0	0	--	0
None	0	2	0	--	2
Total	1	3	0	--	4

[a]Each figure is the number of times in three tests (1957, 1958, and 1959) that there was a statistically significant difference between the protectants compared.

Only in 1957 did seed inoculated and planted immediately produce a yield significantly different from that of the check. In this instance Fusarium spp. resulted in higher yields than those in the check or other inoculum. The reason for the apparent stimulation might have been antibiotic protection against other soil organisms or to stimulation like that produced by gibberellin on certain plants.

In 1957 and 1958 storage before planting resulted in lower yields than from seed inoculated with the same organism and not stored (Table 2). In 1958 even the seed that was not inoculated, but stored before planting, rotted badly, and there was no yield, while in 1957 the non-inoculated seed was practically uninjured. In 1959 only the stored seed inoculated with bacteria differed from the corresponding lot planted immediately.

Table 2 shows also that seed inoculated with bacteria (1957 and 1959) or with Fusarium spp. and bacteria (1957) caused the greatest yield reductions in the stored seed. Fusarium spp. also caused a significant reduction in yield in 1957, while in 1958 none of the stored lots produced any yield, making comparisons impossible.

The extensive seed-piece decay in 1958 as reflected by yields (Table 2) may have been favored by heavy rainfall in the days just after planting. In this year 3.84 inches of rain fell in the period 3 to 8 days, inclusive, after planting. During the same period 1.87 inches of rain fell in 1959 when there was much less decay. However, in 1957 there was only 0.2 inch of precipitation in the first 8 days after planting, and yields from the stored seed were reduced more than in 1959. It must be concluded that differences in inoculum and perhaps other factors besides rainfall were responsible for differences in decay in the three seasons. Since the seed was stored 5 days before planting in 1957 and only 3 days in 1958 and 1959, this may have been partly responsible.

To facilitate the use of the data in Table 1, the protectant chemicals are compared in Table 3 with respect to their relative effectiveness on stored seed. The comparison emphasizes the fact that each of these chemicals may sometimes be superior to the others, and that occasionally they may be worse than nothing. Maneb appears to be superior more often than Agrimycin or a mixture of the two. Agrimycin is effective primarily when seed pieces were inoculated with bacteria, and without inoculation is sometimes injurious. As pointed out by Bonde and Malcolmson (4) this may result from the action of fungi after the suppression of antibiotic bacteria. This may have been true in the present tests, as the decay in much of the seed treated with Agrimycin seemed to be caused by fungi.

Those tests with seed planted immediately after treatment showed few instances of benefit from protectant chemicals (Table 1). Maneb resulted in yields significantly greater than the check in two tests, Agrimycin in one, and the mixture of the two in one test.

These tests corroborate previous ones with protectant chemicals in Minnesota in indicating that such materials are seldom beneficial on seed planted soon after cutting. Storing cut seed seems to be the chief reason for seed-piece decay, and under such conditions decay organisms apparently are naturally present on the tubers in sufficient amounts to cause extensive rotting.

If there is a possibility that cut seed will have to be stored before planting, protectant chemicals may be beneficial. Maneb seems better than the antibiotic used in the present tests. There are a number of materials on the market which are similar to maneb, and might be just as effective.

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APPARENT INACTIVATION OF LATENT A STRAWBERRY VIRUS IN FRAGARIA VESCA PLANTS IN PRELIMINARY TESTS[1]

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Abstract

In preliminary greenhouse tests foliage applications of beta-propiolactone at the rates of 4000 to 8000 ppm appear to have inactivated the latent A strawberry virus in some Fragaria vesca plants. Out of 75 plants originally infected with latent A virus, 5 treated plants, or 6.6%, tested "virus-free" after 8 indexings -- the last made 15 months after treatment.

In 1954 Fulton (2) reported in an abstract the inactivation of Type-2 strawberry virus (1) in infected Robinson strawberry plants by treatment with 2% zinc chloride and zinc sulfate, respectively.

In preliminary tests reported by Miller and Vaughan in 1957 (3), attempts to inactivate the components of the strawberry yellows virus complex in the variety Marshall by spraying the foliage with a large number of chemicals and antibiotics were unsuccessful.

In 1958-1959 the writer attempted to inactivate latent A strawberry virus by spraying the foliage of infected Fragaria vesca plants with beta-propiolactone, which in preliminary tests showed indication of anti-viral activity.

MATERIALS AND METHODS

Beta-propiolactone was used at the rates of 3000 to 8000 ppm in water to treat virus-infected strawberry plants. A small amount of a good spreading agent was added to increase wetting and penetration.

The leaves of a sub-clone of a number of Fragaria vesca (E. Malling clone) plants infected with the latent A virus[3] were thoroughly sprayed with beta-propiolactone when the plants were young and actively growing. In those cases where the material was not phytotoxic (3000 to 5000 ppm), three spray applications were made at approximately weekly intervals before the treated plants were indexed for viruses.

After new leaves developed (usually in 4 to 6 weeks), the sprayed plants were tested for the latent A virus by grafting excised leaf-petioles to alpine hybrid vesca (UC-1) plants infected with the mottle or veinbanding virus. All the sprayed plants which appeared to be "virus-free" after the first indexing were reindexed eight times -- four with a mottle indicator and four with a veinbanding indicator -- to determine whether the latent A virus was still alive. (It is necessary to use a second virus to detect latent A as there are no tangible symptoms of latent A alone in F. vesca. When a second virus (for example, mottle or veinbanding virus) is introduced, the resulting complex produces symptoms that are more severe than those produced by the second virus alone.) The period between the treatment and the last indexing was approximately 15 months.

At least three plants per treatment were indexed. The experiments were repeated two or more times.

As controls, unsprayed F. vesca plants infected with the latent A virus (sub-clone of a known infected plant) were indexed by using alpine hybrid vesca plants infected with the mottle or veinbanding virus.

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[3]All plants treated with beta-propiolactone came from a single infected clone which has been tested on numerous occasions and always found to contain the latent A virus (see Table 1; check). It is presumed therefore, that all units coming from this clone contained the virus in testable amounts.

RESULTS

Results of tests carried on to date are given in Table 1. The data represent the combined total number of plants in all replicates.

Beta-propiolactone at the rates of 4000 to 8000 ppm apparently inactivated the latent A virus in some F. vesca plants.

Table 1. Results of attempts to inactivate the strawberry latent A virus by spraying the foliage of infected Fragaria vesca plants with beta propiolactone, 1958-60.

Concentration (ppm)	Number infected plants treated	Number "virus-free" plants after treatment[a]
0 (check)	10	0
3000	5[b]	0
4000	5[b]	1
5000	17	1
6000	18	1
7000	19	1
8000	11	1
Total	75	5

[a] Foliage "virus-free" as determined by leaf-grafts to alpine hybrid vesca (UC-1) plants infected with the veinbanding or mottle virus; re-indexed four times with the mottle virus indicator and four times with the veinbanding virus indicator. Period between treatment and last indexing approximately 15 months.

[b] Six plants were treated but one died before it could be indexed.

After indexing -- approximately 6 months after treatment -- 51% of the treated plants appeared to be "virus-free." However, the virus was eventually "recovered" after the third to the sixth indexing from the majority of these plants. After the 8th indexing -- some 15 months after treatment (Table 1) -- 6.6% (5/75) were still "virus-free." It would seem that the virus concentration was initially greatly reduced immediately after treatment. However, not all of the virus in the tissues was inactivated and, with the passage of time, a build-up of the virus occurred until the virus became "recoverable."

It might be argued that the latent A virus naturally dies out of some F. vesca plants after becoming infected. However, in sample indexings made of F. vesca plants infected with latent A, the virus has been recovered without exception (see Table 1).

Although only the leaves were treated with the chemical, the fact that 6.6% of the plants were rid of the virus indicates that the chemical must not only have inactivated the virus in the leaves but was also translocated to untreated portions of the plants where it was equally effective.

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A ROOT STAIN DISEASE OF EASTERN WHITE PINE

Charles D. Leaphart

Summary

A root stain disease of eastern white pine, caused by a species of Verticicladiella, killed vigorous trees planted in 1912. The symptoms and manner of spread are discussed. Although this is a destructive disease of eastern white pine, so far it has not killed native tree species growing in diseased centers. The fungus was found in one lodgepole pine root, but no evidence of similar infections in other species has been observed.

An unexplained dying of eastern white pines (Pinus strobus) was reported to the author in 1955. Because these trees were established in two of the more successful plantations in the northern Rockies, their death was of particular interest. Both plantations appeared to be doomed to complete destruction. Therefore, studies were started immediately to find the cause of mortality.

DESCRIPTION OF AREA

Two plantations of eastern white pine, both less than 5 acres in size, about 1/2 mile apart, were established near the Sylvanite Ranger Station in northwestern Montana. The trees were planted in 1912 following the 1910 fire that denuded the area. Several native species that naturally seeded the area are present in the plantations. They include lodgepole pine (P. contorta), western larch (Larix occidentalis), western redcedar (Thuja plicata), and western white pine (P. monticola).

The area is considered a fair site for western larch or western white pine. The soil has a glacial till origin and is quite rocky at the surface. Soil surface layer is shallow to the glacial deposit.

Eastern white pine growth might be considered excellent. Most trees of this species occupy dominant or codominant positions; a few are intermediate. Trees average 60 to 65 feet in height and 8 to 9 inches d.b.h. In the 13-year period (1941-1954), three representative eastern white pines averaged 22 inches' terminal growth per year.

DISEASE SYMPTOMS

One of the first crown symptoms of the root-stain disease is marked reduction in height growth. Examination of dead and dying trees revealed that death occurs a few years after terminal growth reduction starts. To illustrate, two trees that died in 1954 and 1955 had grown about 24 inches in height per year for the 5-year period before growth reduction started. The tree that died in 1954 grew 21 inches in 1952, 16 inches in 1953, and 7 inches in 1954. The one that died in 1955 grew 22 inches in 1953, 8 inches in 1954, and none in 1955. Thus, the time interval between the first noticeable crown symptoms and death was quite short.

Crown discoloration closely follows leader growth reduction. First a slight yellowing appears in the upper crown. By the time leader growth is permanently halted, nearly all of the crown is yellow and most upper crown needles have turned brown. This change is concurrent with tree death, for the root system is completely girdled at or near the root collar by the invading fungus.

Only the root systems were abnormal on examination of diseased trees. A dark chocolate to black-brown stain was found in root sapwood. This stain progressed from some point on the distal end toward the root collar in all infected roots (Fig. 1). Where infection originated in the root was not determined. Root connections or grafts were observed to be one means of transmission from an infected to an uninfected root.

The disease in the roots is characterized by streaks of dark stain (Fig. 1), which coalesce gradually. On the trees studied, an infected root was invariably girdled at some point toward its distal portion. From that girdle, all the root sapwood was stained to the root tips.

The stain progressed quite rapidly in roots in advance of cambial necrosis toward the root collar. Sometimes a root was stained as far as 3 to 4 feet in advance of cambial death. Macroscopically, the cambium in this area of xylem invasion appeared very healthy in all respects. No microscopic sections or isolations were made of bark and cambial tissues at these points

FIGURE 1. Eastern white pine buttress root infected with root stain disease. From a point where the cambium was dead (A), the fungus and associated stain had progressed to (B) under apparently normal cambium.

FIGURE 2. A dominant eastern white pine with the stem peeled to reveal fungus progress. Stain has progressed from the roots up the stem 10 feet. Cambium over the upper 3 feet appeared quite normal. This tree's crown appeared fairly normal.

FIGURE 3. The dead tree in the center is on the edge of a disease center. Although appearing quite normal, the tree to the left has diseased roots. A group of dead trees immediately behind the dead one represents the original infection center.

to detect fungus mycelium. But a fungus could be isolated from any stained xylem -- not from surrounding unstained wood.

Once a tree became infected, even in only one root, its death appeared to be inevitable. A tree with only one uninvaded root might appear quite healthy, but eventually the root collar would be invaded. When this occurred, the fungus spread tangentially to the uninfected root; thus, the diseased tree was girdled and killed.

The stain often proceeds up the main bole of the tree from the buttress roots before the complete girdling at the root collar. Distances the stain extends in the stem above ground line vary (Fig. 2). The stain penetrates the entire depth of sapwood beneath the discolored streaks and, as in roots, it may extend several feet beyond the area of killed cambium.

CAUSAL AGENT

Several fungi have been isolated from the stained xylem. One occurred in slightly more than 90% of the 1955 isolations. Isolations in succeeding years primarily to obtain inoculation material have been equally successful. The fungus produces conidiophores of the Leptographium type[1]. It has been recently identified and placed in the genus Verticicladiella[2].

Although the fungus was easily isolated from stained wood, little or no growth has been obtained on subsequent transfer to agar slant tubes. Therefore, naturally infected root sections from which Verticicladiella sp. was isolated were used in inoculations. Inoculations were made by exposing and wounding selected roots, placing infected root sections on the roots and wrapping them in place, and then replacing the soil around inoculated roots.

So far, at least, one successful inoculation has resulted from these attempts and the fungus was reisolated. Initial progress of the stain from the inoculation appears quite similar to stain developing in naturally infected roots. Both the frequency of isolation and this one inoculation result suggest that this fungus causes the root stain disease. While the results are promising, more study is required for conclusive evidence.

DISCUSSION

Despite the lack of conclusive inoculation experiments, this species of Verticicladiella is without doubt pathogenic on eastern white pine in the plantations described. The disease radiates from a common center, apparently a single inoculum source, suggestive in its manner of spread (Fig. 3) of other root pathogens, for example, Poria weirii Murr. At least five different centers have been observed, several widely separated from each other. All centers are of recent origin, that is, dead, fallen, or standing trees were at least 40 feet tall when killed. The largest center covers about 1/4 acre, and the smallest has only a few infected or dying trees.

Only one native conifer species growing in the plantation has shown signs of susceptibility to the fungus. One infected root of a lodgepole pine tree growing in a badly damaged center was found and the fungus was isolated from it. The stain and nature of fungus spread in that root were similar to those symptoms found in eastern white pine. However, subsequent examinations of this tree have not revealed additional infected roots. Nevertheless, the fact that one native host species was infected suggests that the fungus should receive more study, particularly regarding host susceptibility.

A few other plantations of eastern white pine in the northern Rockies have been examined. The disease has not been found in them. Not all plantings of this species have been visited, however. No reports have been received of tree mortality with the symptoms described here; however, foresters should examine known plantations for evidence of this disease. Reports of such examinations would help in determining the prevalence of this fungus.

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[2]Correspondence January 26, 1960 with Dr. W. B. Kendrick, Research Branch, Science Service, Plant Research Institute, Ottawa, Canada. Dr. Kendrick has not yet assigned a species name to this fungus. He plans to discuss this fungus in a monograph to be published soon.

HEAT TREATMENT ELIMINATES YELLOW DWARF VIRUS FROM SWEETPOTATOES

E. M. Hildebrand and Philip Brierley[1]

Abstract

The terminal growth of most sweetpotato plants in the hot chamber exceeded that of plants held at room temperature and 5 feet of new growth per month was not uncommon. New terminal growth that developed on diseased sweetpotato plants in the 38° C chamber was uniformly symptomless regardless of the variety or viruses originally present; whereas, yellow dwarf or feathery mottle symptoms reappeared usually in a minority of the cuttings taken after 1 month of heat treatment, relatively rarely after 2 months of treatment, and never after 3 months. The 3-month treatment also practically eliminated the leafspot symptoms of the internal cork syndrome, the significance of which remains to be determined by testing other sweetpotato selections.

Heat therapy was first demonstrated 35 years ago when sugarcane was freed of chlorotic streak and sereh disease viruses by hot water treatment. Subsequently, healthy material was salvaged from many virus-diseased plants by hot-water and hot-air treatment (1).

Some viruses are particularly vulnerable to heat and can be eliminated from whole plants by moderate treatments (5), but other viruses thus far have never been eliminated even from the minute shoot tips used in meristem culture. Hot-air treatments commonly employ temperatures of 35° to 43° C. Few plant species survive the higher temperature for more than a few days. Sweetpotatoes -- an exception -- are notably heat tolerant and grow actively for extended periods (months) under conditions that are soon (within weeks) lethal for hydrangeas, chrysanthemums and carnations.

The hot-air treatment of whole diseased sweetpotato plants was used in this study, in an endeavor to eliminate virus from the new terminal growth. More specifically, 38° C was tested for separating the components of feathery mottle virosis, a complex containing three viruses (4) and for determining its effect on the yellow dwarf virus component alone.

MATERIALS AND METHODS

A compartment described earlier (2) for hot-air treatment was modified by ducting the heat to baseboard heaters and by adding an exhaust blower and an air conditioner to combat excess summer heat. Pneumatic-electric relays operated the blower instead of the inside and outside ventilators. The thermostat was set at 38° C, but the recorder showed the temperature actually fluctuated from 35° to 41°. It is difficult to maintain close temperature control in a chamber open to sunlight, but plants appear more tolerant to heat when adequately lighted.

Young growing diseased sweetpotato plants of several varieties were transplanted into 6-inch pots and held at room temperature (80° F) for 1 day before they were moved into the hot room. Terminal growth produced in the hot room was removed at monthly intervals. Only in the first series were the terminals cut into 2-node scions for grafting into healthy indicator plants. When the grafting method proved less efficient in indexing than by the use of rooted cuttings, the former method was supplanted by the latter.

The new growth produced in the hot chamber was cut from the plants, divided into units consisting of two nodes (of which the lower one had the leaf removed), and numbered from the base to the tip. These cuttings were placed in a sand bed for rooting. Within about 6 days the rooted cuttings were transplanted into 4-inch pots for passing through the "flush of growth" (3), which occurs about 4 weeks later, to reveal their health status.

RESULTS AND DISCUSSION

The terminal growth of most sweetpotato plants in the hot chamber exceeded that of plants held at room temperature and 5 feet of new growth per month was not uncommon. The new

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sweetpotato growth that developed in the 38° C room was uniformly symptomless. Yellow dwarf symptoms were lacking on the new growth, but these symptoms reappeared usually in a minority of the cuttings taken from heated plants. The yellow dwarf virus was eliminated whether it occurred alone or as a component of feathery mottle, and regardless of the sweetpotato variety or selection heated. By labeling and recording the numerical sequence of the cuttings taken at monthly intervals, it was soon established that virus invasion always took place at the base of the first new terminal growth.

Table 1 summarizes the results of the first series of 38° C temperature studies on sweetpotato feathery mottle under four headings: 1) feathery mottle (FM), or yellow dwarf (YD); 2) leafspot (LS); 3) healthy (H); and total cuttings (TC).

Table 1. Incidence of symptoms of feathery mottle and of the systemic vein-clearing yellow dwarf viruses in the terminal growth of cuttings taken from initially diseased plants grown in a room held continuously at about 38° C.

Variety or selection	Plant number	Interval (months)	Symptoms in cuttings taken from plants initially infected with[a]: feathery mottle FM	LS	H	TC	yellow dwarf YD	LS	H	TC
Russian	1	1	0	13	7	20				
		2	1	8	6	15				
		3	0	0	26	26				
	2	1	6	4	1	11				
		3	0	0	14	14				
Porto Rico	1	2	1	12	2	15				
		3	0	1	5	6				
	2[b]	1					0	26	3	29
La. IX	1	1	5	15	13	33				
		3	0	1	20	21				
	2	2					7	0	11	18
		3					0	0	16	16
La. I	1	1	1	11	4	16				
		2	0	7	12	19				
	2	1	0	2	22	24				
	3	3					2	0	10	12
La. XXI	1	1					2	0	3	5
Yellow Jersey	1	1					5	0	0	5

[a]Columns 4 and 5 have four headings: 1) feathery mottle (FM) or yellow dwarf (YD), 2)leafspot (LS), 3) healthy (H), and 4) total cuttings (TC).

[b]Plant 2 of Porto Rico containing only the internal cork syndrome included as a check in the yellow dwarf (YD) column.

The effect of the 38° C heat treatment on plant 1 of the Russian variety is shown after exposures of 1, 2, and 3 months. For the first month interval of exposure none of the 20 cuttings were infected with feathery mottle, but 13 were infected with leafspot and 7 were healthy. For the second month there were 1 cutting with feathery mottle, 8 with leafspot and 6 healthy; and for the third month there were none with feathery mottle and none with leafspot; all 26 were healthy.

By way of comparison, Russian plant 2, which was severely infected with feathery mottle, showed after the 1-month interval of exposure 6 cuttings with feathery mottle, 4 with leafspot and 1 healthy. For the third month, this same plant showed all 14 cuttings healthy.

Essentially the same results were obtained for the variety (Porto Rico) and selections (La. IX, La. I) affected with the feathery mottle complex. These results demonstrate that the 38° C heat treatment operates independently on the virus and host by apparently repressing virus multiplication while simultaneously stimulating host growth.

When the plants containing only the yellow dwarf virus component of feathery mottle were subjected to the same 38° C heat treatment, essentially the same results were obtained. For example, for the La. IX selection, the cuttings produced during the second month interval of exposure showed 7 with yellow dwarf, 0 with leafspot, and 11 healthy. For the third month all 16 cuttings from the same plant were healthy. In like manner, heat treatment of the other two selections (La. I and La. XXI) and 1 variety (Yellow Jersey) affected with yellow dwarf alone gave much the same results for the first month interval. From these tests it is apparent that 38° either stops or greatly retards multiplication of yellow dwarf virus whether present in the feathery mottle complex or alone, to account for the elimination of this virus from many of the cuttings from the new growth produced in the hot chamber.

Plant 2 of the Porto Rico variety was free of yellow dwarf, but contained the internal cork syndrome or a mixture of the internal cork and leafspot viruses. When subjected to the 38° C treatment for 1 month practically all the cuttings showed leafspot symptoms, as shown in the table.

It is apparent that by the end of the 3-month interval of exposure of the Russian variety to the heat treatment, the leafspot virus symptom had also been eliminated from the terminal growth as represented by the cuttings. The significance of this observation remains to be determined.

The general effect of heat treatment, at 38° C, apparently is to depress virus multiplication and/or accelerate virus degradation, so that even heat-tolerant viruses are hampered in keeping up with plant growth. The vigorous growth that sweetpotatoes make at 38° lends hope that they might outrun any virus.

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METHODS OF SOIL INFESTATION, WATERING, AND ASSESSING THE DEGREE OF ROOT INFECTION FOR GREENHOUSE IN SITU ECOLOGICAL STUDIES WITH CITRUS PHYTOPHTHORAS

Peter H. Tsao and M. J. Garber[1]

Summary

Mycelia of Phytophthora citrophthora and P. parasitica grown in liquid medium were suitable inoculum materials for artificial soil infestations. Waterlogging the soils periodically favored both normal root growth of citrus seedlings in noninfested soils and severe root infection in infested soils. The healthy root tip count method was a quick and accurate quantitative method for assessing the degree of root infection in short-term experiments. These methods proved reliable and workable, and have greatly facilitated greenhouse studies on in situ ecological factors affecting root infection by these fungi.

Two of the main causal fungi of citrus fibrous root rot in California are Phytophthora citrophthora (R. E. Sm. & E. H. Sm.) Leonian and P. parasitica Dast. (2, 5). As knowledge of the effects of biological and other in situ ecological factors on root infection by these fungi is lacking, studies of these aspects have recently been initiated. Numerous earlier experiments using various methods were unsuccessful due largely to failure to obtain a desired amount of root infection in infested soils. It was found difficult to establish the fungus in the soil by the use of zoospore suspensions. The zoospores, when used in the root-dip inoculation method (4, 5), readily infected the roots but were not subjected to the influence of various soil ecological factors in short-term experiments. In experiments of longer durations (4 to 6 months), the small amount of primary inoculum of zoospores was not sufficient to produce disease severe enough for adequate comparison among treatments. Regular watering of the infested soil (that is, water added when soil appeared dry), even with a high inoculum level, brought about little root infection. Root rot was severe in the continuously saturated soil, but the control seedlings also had reduced root systems due to asphyxiation. It was soon realized that a satisfactory soil infestation method that would allow a uniform amount of inoculum to be established in the soil prior to root infection, a suitable watering method that would provide an optimum soil environment for both root growth and disease, and an adequately accurate method for assessing the degree of root infection in short-term experiments were necessary prerequisites for these intended studies.

Preliminary experiments showed that mycelia of the two Phytophthora spp. were suitable as soil inoculum, provided a correct watering was exercised subsequently. The mycelial inoculum could be produced in large quantities by growing the fungus in a potato-dextrose broth. No sporangia or oospores were produced in the liquid medium, at least during the first 3 weeks of observation. Soil infestation with the mycelial inoculum brought about a desired expression of disease on the seedlings at about 3 to 4 weeks, and the severity of root infection was in direct relation to the amount of inoculum added to the soil. Wager's (10) method of using saucers for waterlogging the soil was satisfactory. Waterlogging the soil periodically favored both root infection in infested soils and normal root growth in noninfested soils. There was little or no sloughing of the root cortex on these control plants. Almost all root tips were healthy with white pointed tips, in contrast to the rotten or blunt ends of many of the infected fibrous roots in the infested soils. Although healthy root tips also were present on the diseased seedlings, the number usually decreased correspondingly with the increase in the amount of mycelium added to the soil. The health of diseased seedlings was expressed by the number of healthy root tips remaining on the roots.

A number of experiments were carried out to test the newly acquired information concerning the mycelial inoculum, the periodic waterlogging, and the healthy root tip count methods. This paper reports the results of these experiments.

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MATERIALS AND METHODS

Organisms: Two isolates of Phytophthora parasitica were used: T64, from a citrus soil in San Diego County, and T89, from sweet orange roots in San Bernardino County. The P. citrophthora isolate used was T52, a lemon bark isolate from Orange County. All three were known to infect citrus fibrous roots. As P. citrophthora succumbs to high summer temperatures in the greenhouse, P. parasitica was used in most of the tests.

Seedlings: The citrus seedlings (sweet orange, Citrus sinensis var. Homosassa) were 2 to 3 months old since seedling. They were 3 to 4 cm in height with 4 to 8 leaves and 12 to 25 fibrous roots. Within each experiment, however, the seedlings were uniform in size.

Soils: Unless otherwise indicated, the soils used were a mixture (1:1) of silt and a clay loam top soil (with pH 6.4 and 6.2-7.0, respectively). The soil mix was steamed (82° C or higher) for 1.5 to 2.5 hours, depending on the volume of soil in the containers. The pH of the soils after steaming was usually 6.3-6.8. A measured amount of soil was added to each clay pot: about 1000 cc (1300-1500 g) for the 5-inch pots and about 500 cc for the 4-inch pots.

Inoculum Production and Soil Infestation: The mycelial inocula for soil infestation were prepared by seeding 100 ml of potato-dextrose broth (per liter: extract from 250 g potato; dextrose, 20 g) in each 32-ounce prescription glass bottle with a 7 mm agar-mycelium plug from the 7 to 15 day old fungus cultures grown on potato-dextrose agar plates. The plugs were taken from the colony margins by means of a cork borer. After the seeded bottles had been incubated vertically at 25±2° C for 22 to 24 hours, they were placed on a reciprocal shaker (making 82 cycles per minute with a 7-cm stroke and a 30-cm radius) for 1.5 to 2 hours to break up the hyphae grown out from the mycelium plugs. The bottles were then incubated horizontally as stationary cultures. After 7 days the fungus had nearly covered the entire medium surface. The mats were harvested from the bottles, pooled, filtered through four layers of cheese cloth and rinsed repeatedly with deionized water on a Büchner funnel to remove any remaining nutrients, and macerated in a small amount of water in a Waring Blendor for 10 to 20 seconds. The mycelial fragment suspension was then mixed thoroughly with a calculated amount of sterile coarse sand and a measured amount of the sand-inoculum was mixed into the soil. The infested soil was repotted with one seedling in each pot. The nonifested soil received sand only. When more than one inoculum level was used (1X, 1/2X, ... 1/10X, and so forth), the sand-inoculum was further mixed with calculated volumes of sand to give the desired dilutions. The inoculum level of 1X consisted of the average amount of inoculum from one culture bottle in each pot of soil.

Watering: Deionized water was used. Before the periodic waterlogging began, all pots were watered regularly for 3 to 4 days; water was added only when the soil appeared dry. The periodic waterlogging was achieved by using a clay saucer (6 inches in diameter and 1 1/4 inches in depth) at the botton of each pot (Fig. 4). Soil saturation during the 3-day waterlogging period in each weekly cycle was accomplished by filling the saucers with water daily during the period, in addition to watering the soil surface. The soil capillary action resulting from subirrigation maintained the soil moisture at a waterlogged condition. Little or no water was added to the saucer on the third waterlogging day. If water remained at the end of that day, it was poured off to allow the soil to dry out the next day. Watering during the subsequent 4 days in the weekly cycle was accomplished by adding a small amount of water only when the soil surface was dry. The use of saucers also prevented dissemination of Phytophthora to the adjacent noninfested control pots.

Healthy Root Tip Count Method for Assessing the Degree of Root Infection: This method was used for experiments of short durations, that is, 4 to 6 weeks. Seedling roots were harvested individually by gently removing the plant and soil in toto from the pots. Adhering soil particles were removed from the roots by a jet of water spray. The root systems were spread out on a piece of dark-colored paper and the numbers of healthy-looking white root tips on each seedling were counted and recorded. Since the surface region of the soil in the pots was often dry during the 4-day "dry" periods in the weekly cycles, and the conditions in this top layer of soil were not favorable for infection, the root tips next to the top 2 to 2.5 cm region of the tap root were excluded in the healthy root tip counts for all plants.

In all tests, each treatment was replicated 6 to 8 times (except Experiment 6, 4 times), and arranged on the greenhouse bench in randomized blocks. Greenhouse air temperatures usually ranged from 22° to 34° C, with occasional higher day temperatures in the summer months.

RESULTS

Experiment 1. The periodic waterlogging method was compared with two other watering methods: regular watering and continuous waterlogging (soil maintained saturated at all times by filling the saucers with water throughout the experiment). Four inoculum levels (0, 1/4X, 1/2X, and 1X) of P. parasitica T64 were used for each of the three watering methods. Saucers accompanied all pots.

The average numbers of healthy root tips per seedling in each of the 12 treatments recorded at 4 weeks are illustrated in Figure 1. With the regular watering method, the control plants in noninfested soils had numerous healthy root tips, but root infection on plants in the infested soils was comparatively poor. With the continuous waterlogging method, root infection was severe on plants in the infested soils; root systems of the control plants, however, suffered from asphyxiation and were also greatly reduced. The cortex on most of the fibrous roots had been sloughed off. Though the symptoms resembled Phytophthora infection, extensive root and soil isolations, using the apple (3, 9) and lemon (3, 7) techniques, respectively, revealed no Phytophthora.

Only with the periodic waterlogging method were the root growth of plants in noninfested soils and root infection on plants in infested soils both satisfactory. With all three inoculum levels tested a higher percentage of reduction in the number of healthy root tips was obtained with the periodic waterlogging method than with either of the other two methods (Fig. 1).

Experiment 2. The methods performed well in a study on the influence of certain soil factors on citrus root infection by P. parasitica (6). The amount of root infection was different in two infested soils, the "furrow-bottom" and "under-tree" soils, both previously steamed and almost identical in physical properties but differing in pH and certain other soil chemical properties. The reductions in numbers of healthy root tips on plants in these two soils at 4 weeks were 48% and 5%, respectively, with the inoculum level of 1/10X; and 81% and 38%, respectively, with that of 1X (Table 1).

Table 1. Comparison of the degrees of root infection in two different soils infested with P. parasitica.

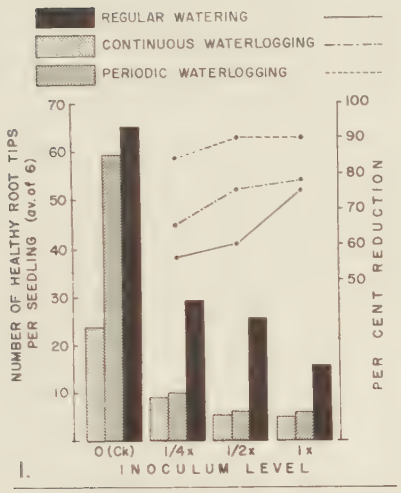
	Number healthy root tips per seedling (average of 7)		
	Inoculum levels		
Soil	0	1/10X	1X
Furrow-bottom	73.1	38.3	13.6
	(significant quadratic relationship at 0.001 level)		
Under-tree	62.6	59.6	38.7
	(significant linear relationship at 0.001 level)		

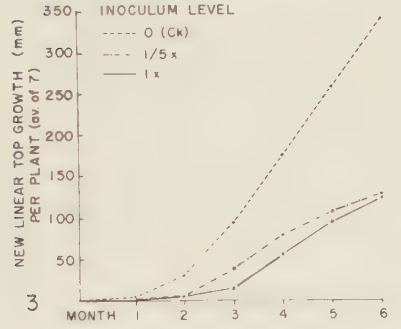
Experiment 3. The methods, along with the serial dilution end-point method for estimating Phytophthora disease potentials (7), were tested in a study on the effect of soil pH on citrus root infection by P. parasitica (8). The data of only one of the experiments are briefly given here for the purpose of illustrating the efficacy of the methods. The degree of root infection on plants in soils of five different pH levels differed, and the percentage reductions in the number of healthy root tips at 6 weeks agreed closely with the Phytophthora Disease Potential Indices (7) of the five infested soils (Table 2). The details of the work will be reported in a future paper.

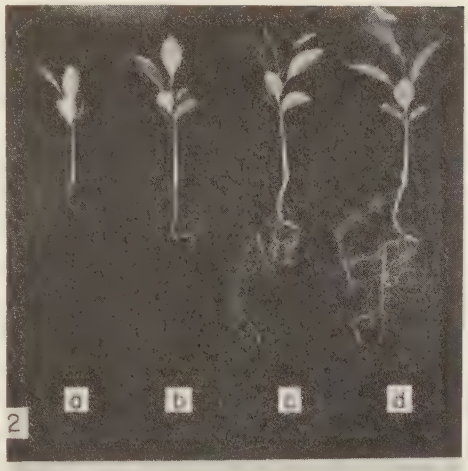
Table 2. Comparison of the degrees of root infection in soils of five pH levels infested with P. parasitica.

Soil	Soil pH		DPI[a]		Number healthy root tips per seedling (average of 7)		
	at transplant	at harvest	at transplant	at harvest	noninfested soil	infested soil[b]	Reduction
1	4.2	4.7	0	0	85.2[c]	70.4	17.4% (ns)
2	4.8	5.3	1.5	2	80.5	57.2	28.9% (**)
3	5.9	6.4	8	8	73.4	28.0	61.9% (***)
4	7.0	7.1	8	4	77.0	34.0	55.8% (***)
5	7.4	7.4	8	4	75.6	45.0	40.5% (**)

[a]Of the infested soils. Definition of DPI (Disease Potential Index), see (7).
[b]Infested with P. parasitica T89 (1X per 1000 cc of soil).
[c]Differences not significant between any two of the five figures in this column.







FIGURES 1 - 4. 1 -- Comparison of the three watering methods. With all three watering methods the check was significantly (0.001 level) different from the inoculated materials. A significant (0.01 level) linear relationship was found between inoculum levels and root damage with the regular watering method only.

2 -- Root systems of representative seedlings in four soils containing different relative amounts of natural inoculum of P. parasitica: (a) the original soil, (b) 1/4 original, (c) 1/16 original, and (d) the steamed soil (control).

3 -- Top growth of seedlings in soils of three inoculum levels of P. parasitica.

4 -- Seedlings after 10 months in noninfested soil (A) and in soils artificially infested with P. parasitica (B), both receiving the periodic waterlogging treatment.

Experiment 4. The periodic waterlogging and the healthy root tip count methods were tested with a field soil naturally infested with P. parasitica. A portion of the soil was steam-sterilized for 1.5 hours and used as the diluent for the original soil to provide four soils containing different relative amounts of the natural Phytophthora inoculum: (a) the original, not diluted, (b) one-fourth original, (c) one-sixteenth original, and (d) the steamed soil as the control. A seedling was transplanted into a 5-inch pot containing a uniform amount of each soil, with each treatment replicated eight times. The Phytophthora Disease Potential Indices of the four soils were found to be 8, 2, 0.5 and 0, respectively (7). Upon harvesting at 1 month, the average numbers of healthy root tips per plant in the four soils were 1.5, 9.9, 29.0, and 58.4, respectively, with significant linear relationship at 0.05 level (Fig. 2).

Experiment 5. In this experiment the methods were tested with P. citrophthora T52. The experiment was performed in the months of January and February when the air temperature was seldom above 30° C, the maximum temperature for the in vitro growth of the fungus (1, 9). Three inoculum levels (0, 1/4X, and 1X) were used, all receiving periodic waterlogging treatment and replicated six times each. The average numbers of healthy root tips per seedling at the three inoculum levels at 4 weeks were 42.3, 31.7, and 19.0, respectively, with significant linear relationship at 0.001 level.

Experiment 6. As all the previous five experiments had durations of 4 to 6 weeks, a single test was run to see whether the methods were applicable in tests of a shorter duration, 3 weeks. P. parasitica T64 and four inoculum levels (0, 1/4X, 1/2X, and 1X) were used. The average numbers of healthy root tips per seedling at the four inoculum levels at 3 weeks were 28.0, 15.0, 4.8, and 4.5, respectively, with significant quadratic relationship at 0.01 level.

Experiment 7. The periodic waterlogging method was used with success in an experiment of a 10-month duration. The plants in noninfested soil attained a height of 63 cm (average of five replicates). The reduction in height on plants grown in soils infested with 1X inoculum level of P. parasitica T64 was over 75% (Fig. 4), and the reduction in dry weight of tops and roots was over 80%.

Experiment 8. The periodic waterlogging method was again tested in a long-term experiment (6 months), using three inoculum levels (0, 1/5X, and 1X) of P. parasitica T89. A steam-sterilized field soil (loamy sand, pH 7.9 after steaming) was used for the experiment. The shoot apex of each seedling was marked with India ink for the monthly height measurements of subsequent new growth. The top was allowed to grow with one single shoot by removing, whenever necessary, the tender growth from the axillary buds except that at the apex. From the monthly height measurement data (Fig. 3), it appeared that the 1/5X and 1X inoculum levels produced different amounts of disease during the third, fourth, and fifth months. However, at 6 months the top heights at these two inoculum levels were nearly identical; the fungus at the 1/5X inoculum level had presumably increased its disease potential during the long experiment. When compared with the control, the reductions in height at 6 months at these two inoculum levels were 61% and 63%, respectively. The reductions in dry weight at these two inoculum levels were also similar: for the tops and roots, respectively; at the 1/5X level, 59% and 62%, and at the 1X level, 63% and 64%.

DISCUSSION

The commonly employed root-dip inoculation method by the use of Phytophthora zoospore suspension (4, 5), which insures quick infection under almost any environmental conditions, is an excellent inoculation method for certain types of studies, such as testing for pathogenicity, screening for resistance, and so forth. However, its very meritorious features render it unsuitable as the inoculation method for various in situ ecological studies, such as the influence of soil microbial antagonists, soil temperature, soil moisture, soil reaction, soil type, and soil nutrient on the growth and infection of citrus Phytophthoras in the soil. In addition, unlike the mycelial inoculum which is present in the soil throughout the relatively short experiment, the initial zoospore inoculum is present on or near the roots only at inoculation time. The secondary inocula in the soil will, therefore, be dependent largely on the scope of the primary infection immediately after inoculation. If the host factor is constant, the amount of root rot is then a result of interactions between, or a function of, the inoculum density of the fungus in the soil and the soil environment. Non-uniformity of the inoculum density would therefore complicate the environmental factors being studied, and cause greater variability in results among the replicates. The results of our experiments presented here showed that the use of Phytophthora mycelia could provide uniform amounts of inoculum at many levels, and the premixing of the inoculum with sand facilitates accurate measurement of the inoculum and thorough mixing of the sand-inoculum into the soil. Furthermore, various desired, sometimes even predictable, amounts of root infection could be obtained by using various inoculum levels. In most soil ecological experiments, because of the large scale, one is usually forced to use only one inoculum level. Predictability of results by using a suitable inoculum level, so as to obtain a series of greatest possible differences in the degree of root infection among the various treatments, is therefore of great importance. In tests where soil factors which would suppress root infection are studied (such as soil antagonists and soil fungicides) a high degree of root infection in the nontreated control is desired. Similarly, in tests where soil factors which would augment root infection are studied (such as in studies of soil nutrition and of additive or synergistic effects of soil microorganisms), a relatively lower degree of root infection in the non-

treated control is desired. The use of mycelial inoculum enables one to adapt the suitable inoculum levels to the nature of the experiments.

In all experiments performed, seedlings in noninfested soils produced a great amount of root growth under the periodic waterlogging conditions. The period of 4 days of sub-saturated soil condition in the weekly cycles apparently provided sufficient aeration in the soil for normal root growth. Root infection on seedlings in infested soils receiving the same watering treatment was, meanwhile, severe. The periodic waterlogging treatment -- by presumably providing optimal conditions for sporangial production and zoospore liberation and dissemination during each weekly cycle -- allowed repeated onslaughts of root infection by the fungus during the entire course of the experiment.

In experiments of long duration, prolonged periodic waterlogging of the soil by the use of the clay saucers could cause substantial leaching, and thus loss of certain soluble chemicals from the soil. In Experiment 8 the pH of the soil was changed in 6 months from 7.9 to 7.2. The use of saucers for waterlogging the soil may not be desirable for long-term experiments where soil chemical factors are studied.

Citrus spp. are slow-growing plants. The conventional method of comparing heights and dry weights for assessing disease, such as used in Experiments 7 and 8, requires at least 4 or 5 months to obtain reliable data and detectable differences among the treatments studied. Our experiments showed that the amount of disease could be assessed within a short period of 4 to 6 weeks by the use of the healthy root tip count method. Along with the serial dilution end-point method for estimating Phytophthora disease potentials (7), this quantitative method has greatly facilitated the ecological studies with these fungi. Dr. S. D. Garrett informed the senior author in a personal communication that a similar root tip count method was used with good success by him and his colleagues in Britain and Australia in their Pythium work with pine seedlings.

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RELATIVE RESISTANCE OF SOME CHESTNUT SPECIES AND HYBRIDS INOCULATED WITH THE BLIGHT FUNGUS

Frederick H. Berry[1]

In 1957 seedling trees of four species of chestnut (Castanea) and various types of chestnut hybrids in an experimental plot near Glenn Dale, Maryland were inoculated with the blight fungus, Endothia parasitica, to determine their relative resistance to blight. The seedling trees were grown from pure seed of the following chestnut species: American chestnut (C. dentata), European chestnut (C. sativa), Henry chinkapin (C. henryi), and Chinese chestnut (C. mollissima). The hybrid chestnut trees were grown from seed resulting from controlled pollinations between trees of the pure species.

Table 1. Relative resistance of species and hybrids of Castanea as indicated by results 15 months after inoculation.

Species or hybrid	Number trees inoculated	% trees infected	Average area of cankers (sq. in.)	Number trees killed
Species:				
dentata	14	93	21	0
sativa	16	69	13	2
henryi	11	64	10	0
mollissima	143	29	7	0
Hybrid:				
mollissima x dentata	41	85	20	0
(mollissima x dentata) F_2	29	69	11	1
mollissima x (mollissima x dentata)	28	68	11	0
mollissima x (crenata x dentata)	1	--	--	1
mollissima x alnifolia	1	100	2	0
mollissima x henryi	5	100	10	0
(mollissima x dentata) x henryi	7	100	23	1
mollissima x sativa	3	67	14	0
mollissima x seguinii	3	67	9	0
(mollissima x (mollissima x dentata)) x (mollissima x dentata)	3	67	12	0
mollissima x ((mollissima x dentata) x mollissima)	3	67	9	0
mollissima x crenata	2	0	--	--
mollissima x pumila	4	0	--	--
((mollissima x dentata) x mollissima) x henryi	1	0	--	--
crenata x (pumila x crenata)	2	0	--	--

Approximately 380 trees from 5 to 10 years old were given more than 680 inoculations. To insure infection each tree trunk was usually inoculated twice between ground level and 4 feet above. The east side of the trunk was inoculated, since it had been reported that the frequency of blight infection from inoculations was greatest there[2]. If both inoculations produced cankers, the dimensions of the larger one were measured.

The chestnut blight fungus was isolated in 1957 from a canker on an American chestnut. From a single spore on the original plate culture sub-cultures were grown on corn meal agar in Petri dishes. The inoculum was taken from these subcultures and consisted of a mass of spores, mycelia, and substrate.

[1]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

[2]Clapper, Russell B. 1952. Relative blight resistance of some chestnut species and hybrids. J. Forestry 50: 453-455.

The bark at the point of inoculation was wiped with cotton moistened with alcohol. Uniform incisions (punch holes) were made through the bark and a short distance into the wood by a cork borer 1/4 inch in diameter. A piece of inoculum, slightly smaller than the bore of the punch hole, was picked up with an alcohol-sterilized dissecting needle and placed in the wound. A square of surgical adhesive tape was placed over each hole. Punch holes for the controls were left empty but were closed with tape. None of the 51 control punch holes developed cankers.

The inoculations were made within about 2 weeks, beginning July 23. Cankers were measured 15 months after inoculations were made. The area of a canker was obtained from the product of its width times its length, and measured to the nearest quarter of an inch.

The results from inoculating the four chestnut species and various groups of chestnut hybrids are shown in Table 1.

Although none of the American chestnut trees had been killed by the blight fungus 15 months after inoculation, all but one were infected. European chestnut was slightly more susceptible to the blight fungus than the Henry chinkapin. As expected, Chinese chestnut was the most resistant species, but 29% of the trees were infected.

Crosses between Chinese and American chestnut trees have been made in order to combine the blight-resistance of the Chinese species with the upright growth habit of the American species. First-generation hybrids of this cross were very susceptible to the blight fungus, 85% of them being infected with an average of 20 square inches of cankered area. Backcrosses of these hybrids to the Chinese chestnut parent were more resistant. Only 68% of these hybrids were infected, with an average of 11 square inches of cankered area. Second-generation Chinese x American chestnut hybrids also inoculated with the blight fungus were considerably more resistant than the first-generation trees; 69% were infected and the average cankered area was 11 square inches.

Other chestnut hybrids of various combinations were inoculated, but the number of trees inoculated was rather limited and no attempt will be made to draw any conclusions from the results of these inoculations.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE,
UNITED STATES DEPARTMENT OF AGRICULTURE

DISEASES OF CUT GREENERY IN OREGON[1]

Robert C. Lambe

The cutting, packing, and shipping of cut greenery constitutes a sizable specialty crop industry in the Pacific Northwest. Carloads of sword fern, salal, and huckleberry are shipped annually for use by retail florists all over the country. All of these plants are subject to diseases which may be initiated in the forests or in the coastal native stands and may continue to develop while materials are in storage and in transit. Considerable losses from infectious diseases occur annually. This study was conducted to determine the cause of losses of sword fern, salal, and huckleberry in order to plan suitable control programs.

LEAF SPOT OF SWORD FERN

Fronds of sword fern, (Polystichum munitum), growing in coastal forests become infected by the fungus Taphrina faulliana Mix (4) in the summer. Infections usually are present only as small white to light green spots on the pinnae (Fig. 1) but occasionally infected areas, which may be 5 mm or more, become distorted and slightly curled. In some instances the rachis may also become infected. Asci of the fungus have been observed on both surfaces of infected pinnae.

Infected areas rapidly decompose and become watery. Tissue isolations from the margin of such spots have consistently yielded a yellow bacterium. Eventually the leaf spots become necrotic and most of them drop out (Fig. 2). Isolations made in the fall from such necrotic spots that are retained by the pinnae have consistently yielded fungi. These have been identified as Phyllosticta sp. and Phomopsis sp. Observations of and isolations from diseased pinnae suggest that the brown spots observed on fronds in storage are caused by secondary bacteria and fungi that invade Taphrina induced spots.

POWDERY MILDEW ON SALAL

Powdery mildew of salal (Gaultheria shallon) caused by Microsphaera sp. is a very serious problem of the greenery industry. Symptoms of the disease are localized or general yellowing apparent from the upper surface of leaves and a white powdery or brown necrotic spotting of the lower surface (Fig. 3). Areas of mildew sporulation may be small or large. As the disease will develop and seriously damage large areas of leaves after the plant material has been packaged and put in cold storage at approximately 34° F, it is surprising that powdery mildew has not been reported before.

PHYLLOSTICTA LEAF SPOT OF SALAL

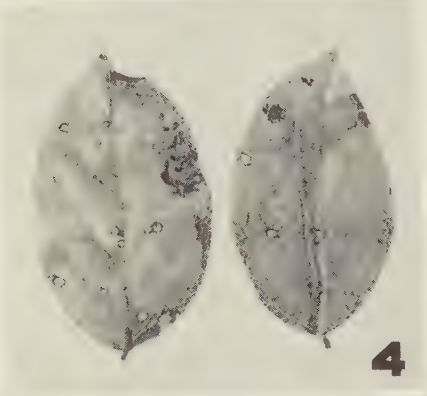
Leaves of salal are severely damaged by the leaf spot fungus Phyllosticta gaultheriae Ell. & Ev. (1, 2). In natural habitats the spots are subcircular, brown, have pale centers, and measure a few mm in diameter (Fig. 4). Pycnidia of the fungus are present in the centers of the spots. These spots have not been observed to enlarge after leaves have been placed in cold storage.

When salal branches are picked in the spring before new growth has appeared, and in the fall after new growth has had no opportunity to harden, severe spotting of leaves occurs in cold storage (Fig. 5). New infections may appear only as water-soaked or pinhead spots on receipt at receiving plants, but will enlarge and eventually coalesce under favorable storage conditions. Apparently healthy leaves will become covered with necrotic spots within 48 hours after they are placed in cold storage and held at approximately 34° F. Often the first and most serious spotting appears on leaves in the center of a bundle of salal. The fungus can be isolated readily from necrotic spots on leaves and also from lesions on stems.

VENTURIA LEAF SPOT OF HUCKLEBERRY

Huckleberries (Vaccinium ovatum) growing in coastal areas of Oregon are commonly infected by Venturia vaccinii Ell. & Ev. (3) which causes necrotic leaf spots. These spots are usually marginal, but may involve large portions of affected leaves (Fig. 6). In necrotic areas of at-

[1]Supported by a grant from Callison's Incorporated, Seattle, Washington.



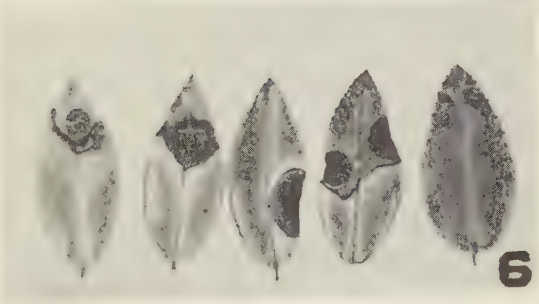


FIGURE 1. Sword fern with infections of Taphrina. (collected near Eugene, Oregon)

FIGURE 2. Sword fern with advanced leaf spot infections. (collected near Chehalis, Washington)

FIGURE 3. Salal leaf with mildew symptoms -- right; healthy -- left.

FIGURE 4. Salal leaves with Phyllosticta leaf spot as it occurs in the forest. (collected near Eugene, Oregon)

FIGURE 5. Salal leaves with Phyllosticta leaf spot as it occurs in storage. (collected near Eugene, Oregon)

FIGURE 6. Huckleberry leaves with Venturia leaf spot infection. (collected near Coos Bay, Oregon)

Photographs by H. H. Millsap

tached leaves, perithecia of the fungus are formed which are visible as a cluster of small black bodies. These necrotic areas have not been observed to enlarge or spread to healthy material in storage, but are nevertheless undesirable because of their appearance.

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EYE SPOT OF LEMON GRASS IN GUATEMALA

Eugenio Schieber and Antonio Sanchez

Eye spot of lemon grass (Cymbopogon citratus), incited by Helminthosporium sacchari (Breda de Haan) Butler, was found in plantations of lemon grass located on the pacific coast of Guatemala. Lemon grass is an important crop for this country, since Guatemala is one of the world leaders in production of essential oil from this grass. This appears to be the first report of eye spot disease in the area. Specimens were collected in the finca Rio Seco, Departamento de Escuintla at an elevation of 90 feet above sea level. Disease development is favored by high temperature and humidity, which prevails in the region parallel to the pacific coast where lemon grass is cultivated. The disease is also known as Florida ringspot since reported from there in 1941[1]. Spots develop on the leaves and are elongate with centers first of a brownish color later turning into a straw color surrounded by a halo. Spots may be found also on culms and sheaths. As described by Sprague[2], the fungus in culture produced conidiophores with bulbous bases, spores brown, curved, fusiform, 3 to 9 septate. The Guatemalan isolate had from 6 to 9 septa.

The areas in Guatemala where lemon grass has been cultivated for several years have never been fertilized. This is of interest since the relation of nitrogen and phosphorous to disease development has been reported in the literature.

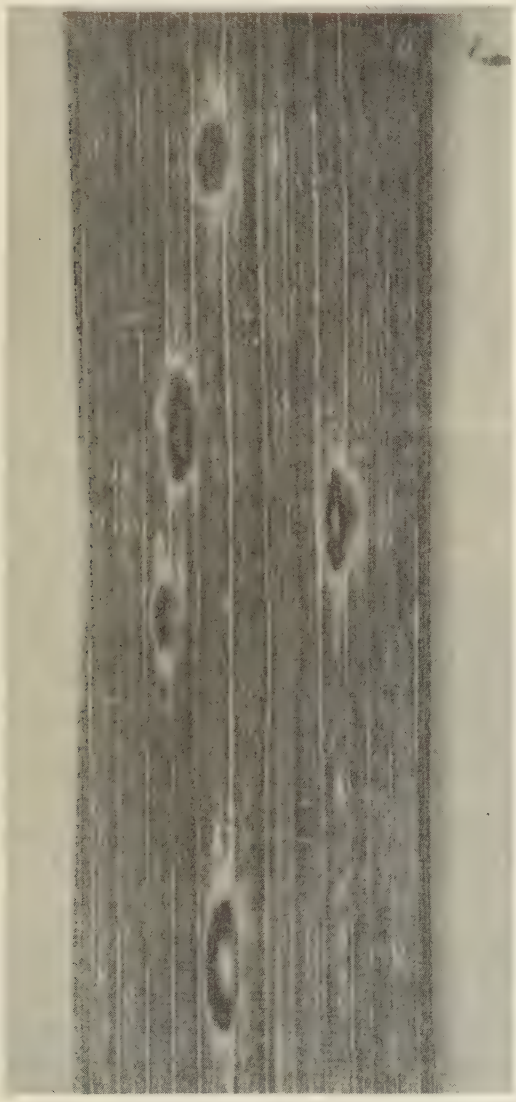

FIGURE 1. Disease symptoms on the upper side of lemon grass leaf.

INSTITUTO AGROPECUARIO NACIONAL, GUATEMALA, GUATEMALA C. A.

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NEMATODES ON COFFEE IN GUATEMALA

Eugenio Schieber and Oscar Nery Sosa[1]

In Guatemala and other Central American countries coffee comprises a very important agricultural crop. One of the problems that has been only slightly investigated in the area is that of nematodes attacking the coffee plant. In the western hemisphere nematodes have been known attacking coffee (Coffea arabica) since Goeldi in 1887 observed a root-knot nematode on coffee in Brazil, which he named Meloidogyne exigua Goeldi (1, 2). This species was first observed by Jobert, in 1878, as a cause of a malady of coffee roots in Brazil. Recently Whitehead (5) reported M. africana n. sp. as a new species found in roots of C. arabica collected in Kenya, Africa. Since Zimmerman (6) reported several species associated with the coffee roots, Pratylenchus coffea Sher & Allen is now recognized as an important plant parasite distributed in many countries, not only on coffee but other hosts.

This report is based on a survey started in Guatemala in November of 1959 and continued during 1960. Parasitic nematodes were found in surveys made in three main coffee areas of Guatemala. These are the important Southwest coast, the Tumbador area and the Southeastern Santa Rosa area. In all plantations surveyed coffee nurseries were affected most with nematodes. In the Southwestern area also mature coffee trees were found to be severely affected. Identifications were made from soil and coffee roots collected at finca "Los Brillantes," "Chocola," and "Las Luces" of Western Guatemala. Soil samples were processed in the laboratory using the Baermann funnel technique. The females were extracted directly from dissected coffee roots. The technique described by Taylor, et al. (4) was used for the identification of Meloidogyne exigua.

Two species of two genera of plant parasitic nematodes were identified in this preliminary survey, namely, Meloidogyne exigua and Pratylenchus coffeae. This appears to be the first report for Guatemala.

In January of 1957, from coffee roots collected in Guatemala G. Thorne of the Plant Pathology Department, University of Wisconsin tentatively identified M. inornata (Lordello 1956). This species was reported by Lordello (3) as a serious pest of soybeans in Brazil. However, the writers were not able to confirm M. inornata in this preliminary survey.

FIGURE 1. Symptoms of nematode attack in young coffee seedlings. Left -- susceptible coffee seedlings. Right -- a healthy plant.

[1]Acknowledgment is given to Pedro Escobar for helping with the field work, and to Guillermo Lucero for preparation of the photographs.

FIGURE 2. Symptoms of nematode attack on roots of mature coffee plants showing the typical galls.

Symptoms produced in the coffee plant by the interaction of both nematodes identified vary according to the age of the plants. In the nurseries young plants show different symptoms (Fig. 1). Roots develop abnormally and plants can be pulled out very easily. The most characteristic symptoms are poor growth of seedlings and leaves turning yellow to brown. Coffee roots of older trees (8 to 20 years) showed galls of different sizes located in groups along the roots and containing large number of nematodes in all stages of development (Fig. 2). These galls attain sizes up to 25 mm. Aerial symptoms of the mature plant include wilting of the leaves, which later turn yellow brown. The trunks of the trees become loose. Affected mature plants suffer mostly during the dry season.

Preliminary studies indicate that there is a difference in relation to resistance between species of coffee. Coffea arabica var. arabica, C. arabica var. bourbon, C. arabica var. pache are very susceptible to these species of nematodes; Coffea robusta, on the other hand, shows a high degree of resistance.

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AN IMPROVED METHOD OF DISINFECTING BARLEY SEED OF USTILAGO NUDA[1]

Donald J. Morton, Irvin K. Hagen, and Everett Tool[2]

Summary

A device was perfected for disinfecting barley seed of Ustilago nuda by the anaerobic method. Soaking, disinfecting, and drying are completed in one container. Two separate experiments utilizing 12 different seed samples demonstrated that the procedure gives excellent control of loose smut without impairing seed germinability.

Therapeutic treatments for barley seed infected with Ustilago nuda (Jens.) Rostr. have been common since Jensen in 1888 (4) showed that the loose smuts of wheat and barley can be eliminated by soaking seed in hot water. A modern technique is to place grain first in a water soak for 5 hours, next in hot water (126° F) for 11 minutes, and finally in cold water to stop the effect of the hot water (5). This method has the disadvantages of being very exacting, requiring refined equipment and appreciably reducing germinability.

Tyner and Russell reported in a series of publications (8-12) that loose smut in barley can be controlled by soaking seed for 48 hours in a 0.2% suspension of Spergon-SL (tetrachloro-para-benzoquinone [= 95% chloranil] in water at 72° F, or in water alone for 80 hours at 66°, 65 hours at 72°, 55 hours at 76°, or 35 hours at 86°. Odorous decomposition products were present after the water treatments, but not after the Spergon treatments. The chemical caused some seed mortality, however. Evidence suggested that a shorter immersion would be effective if the seed were treated in closed containers, but it was believed that growers would find it more convenient to apply the water soak or Spergon treatments in open tubs or tanks. It was pointed out that a difficulty inherent in any of these soak treatments is the need for rapid drying of treated grain.

Hebert (1) disinfected seed by soaking in airtight containers and concluded that the length of treatment needed is inversely related to the temperature of the water. Germination was not significantly reduced by his treatments. Further studies (2) indicated that the principal factor in disinfecting grain by this method is the removal of free oxygen from the closed container by respiration of the wet seed, since the fungus is unable to survive anaerobic conditions. He recommended (3) soaking seed 2 to 4 hours in water followed by storage in an airtight container for a length of time that varied with the temperature; 70 hours at 70° F, 60 hours at 75°, 50 hours at 80°, 40 hours at 85°, and 30 hours at 90°. Canning retorts, steel drums with clamp-on lids, plastic bags, and open drums covered with plastic sheets were all satisfactory airtight containers. The treated grain was either planted immediately or dried and stored for later planting.

The anaerobic treatment appears to be superior to the hot-water treatment in that temperature regulation is not critical and seed viability is not lessened appreciably. It does, however, necessitate soaking, treating, and drying seed, and each of these steps has required separate handling and facilities. To improve the procedure, a method was developed whereby seed may be soaked, disinfected, and dried in one unit.

MATERIALS AND METHODS

A treater was built based upon Hebert's suggestion (3) that an open steel drum covered with a plastic sheet may be an effective airtight container. A 55-gallon drum was fitted with a small valve for draining, and a large bottom opening (4-inch diameter) that can be sealed airtight or attached to an air blower for drying. A false bottom constructed for the inside of the drum consists of window screening supported by heavy wire mesh mounted on six wooden supports. Each support is 3/4 inch wide and 3 inches high, and they are spaced as radii under the circular piece of wire. Additional wooden supports connect the ends of the radial pieces, and this arrange-

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[1]Published with the approval of the Director, North Dakota Agricultural Experiment Station. Supported in part by a grant from the Malting Barley Improvement Association.

[2]Assistant Plant Pathologist, North Dakota Agricultural Experiment Station, Fargo, and Deputy Commissioners, North Dakota State Seed Department, Fargo.

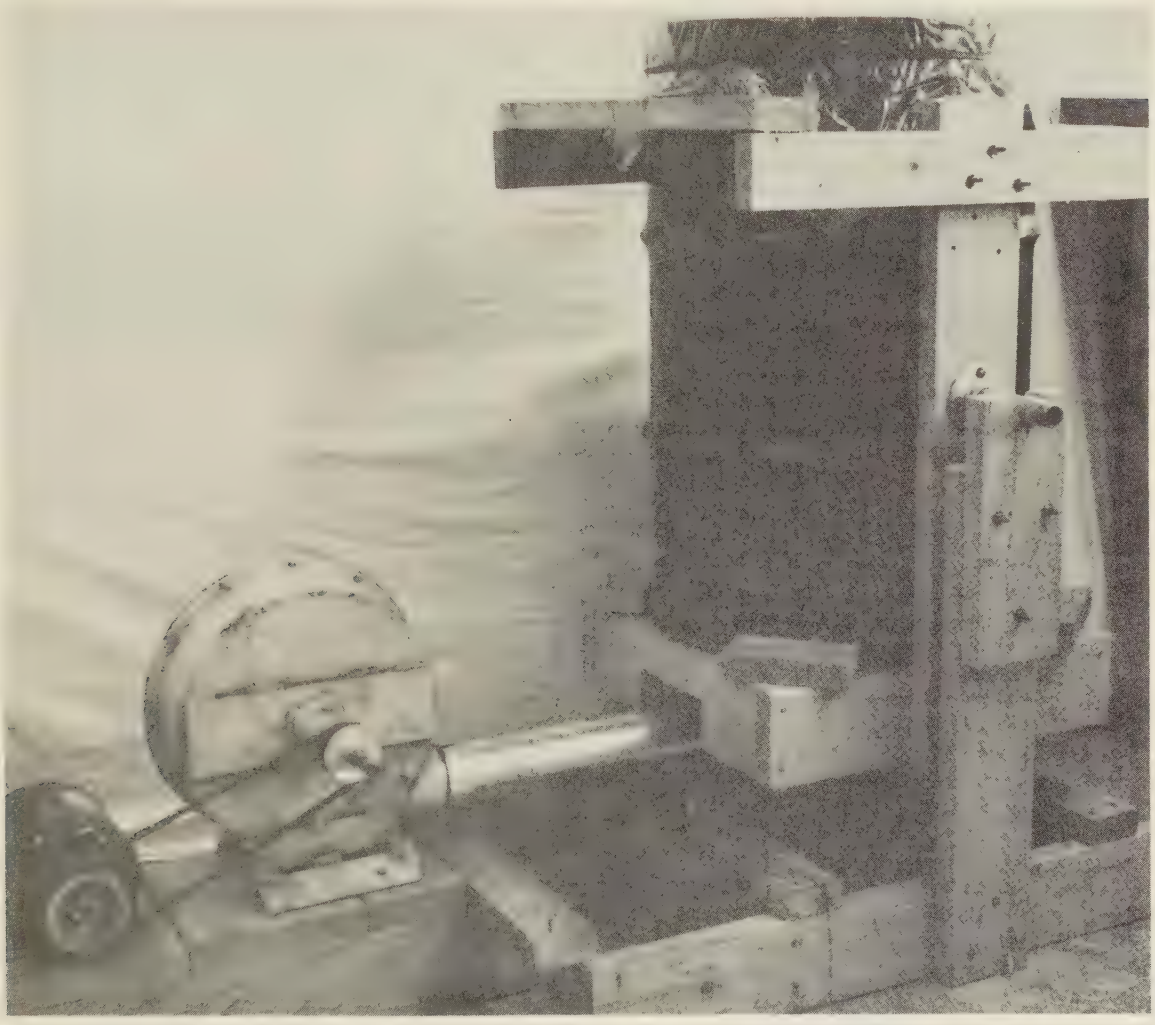

FIGURE 1. Equipment used in disinfecting barley seed. An air blower driven by an electric motor is connected by a 4-inch pipe to the 55-gallon drum on the right. The plastic seal covering the drum is removed when the blower is in operation.

ment has been found effective both for draining and for drying the grain.

A procedure for disinfecting seed was developed. Grain is added until the drum is about 3/4 full, and water at about 70° F is poured in and the seed allowed to soak for 2 to 4 hours. Then the small valve is opened and excess water drained out. The valve is then closed, and a plastic sheet is fastened over the top of the drum with a strong elastic. The seed is kept thus for 65 to 70 hours. A ballooning of the plastic sheet from gas production attests that the seal is airtight. When the treatment is finished, the plastic sheet is removed and the bottom 4-inch seal is opened and connected to an air blower which moves sufficient air that a light breeze can be felt rising from the surface of the wet seed. Grain has been dried to 14.4% moisture content by running the blower for 8 hours on each of 2 or 3 successive days. Seed decay and sprouting were completely inhibited. The drum is mounted on a swivel for emptying the dried seed (Fig. 1).

Two experimental trials have been run with the above method. In the first, barley seed with 6% loose smut as determined by the embryo test used at the North Dakota State Seed Department (6) was treated and compared with untreated samples of the same seed lot. Grain was planted in 6-inch greenhouse pots and grown to maturity. The number of smutted heads that developed was used as a measure of the treatment's effectiveness. Also, seed was planted in greenhouse flats so that third nodes (crown nodes) could be removed from seedlings and examined

for fungus infection, as described by Morton (7). About 500 seeds were planted and evaluated for both treated and untreated seed samples with each method of testing.

In the second trial, 11 samples of barley seed with different percentages of U. nuda infection were wrapped in individual cheesecloth bags and placed in the center of the drum, and waste barley was used for fill. Samples were treated in this manner so as to test a variety of seed lots without using the quantity of seed and length of time that would be needed if lots were treated separately. Seed was soaked and treated as previously described, except that bags were removed for drying because they hindered air movement. Seed disinfection was determined by the seedling test mentioned earlier, and about 100 seed of each lot were tested and compared with untreated lots. Also, some samples were grown to maturity in the field and 800 heads per sample were examined for smut. The percentages of initial seed infection in the samples were determined by the North Dakota State Seed Department to be as follows: Lot 1-11.6%; lot 2-20.4%; lot 3-22.1%; lot 4-22.0%; lot 5-28.2%; lot 6-23.5%; lot 7-7.7%; lot 8-8.6%; lot 9-5.1%; lot 10-12.0%; and lot 11-35.0%.

RESULTS

In the first test (Table 1) germination percentage was not lowered by the treatment, and therapy of infected seed appears to have been virtually complete as determined by both greenhouse seedling and greenhouse maturity evaluations. The lone exception was one smutted head found in the maturity test.

Table 1. Effect of the anaerobic treatment on seed infection as determined by greenhouse seedling and greenhouse maturity evaluations. The original percentage of infected seed was estimated to be 6.0.

	% germination	Number seedlings tested	% seedlings infected	Number heads counted	% smutted heads
Untreated	93.5	500	6.6	573	5.9
Treated	94.6	500	0.0	549	0.2

Table 2. Effect of anaerobic treatment on seed infection as determined by the seedling evaluation technique and field maturity tests.

Sample number	% initial seed infection	Treatment	Number seedlings tested	% seedlings infected	% smutted heads
1	11.6	Untreated	97	8.6	11.7
		Treated	106	0.0	0.0
2	20.4	Untreated	84	19.1	14.6
		Treated	96	0.0	0.0
3	22.1	Untreated	98	20.5	20.9
		Treated	109	0.0	0.0
4	22.0	Untreated	80	16.0	-
		Treated	91	0.0	-
5	28.2	Untreated	121	22.3	21.4
		Treated	95	0.0	0.0
6	23.5	Untreated	104	20.3	-
		Treated	88	0.0	-
7	7.7	Untreated	96	6.2	5.2
		Treated	104	0.0	0.0
8	8.6	Untreated	92	8.4	7.7
		Treated	95	0.0	0.0
9	5.1	Untreated	93	4.4	4.2
		Treated	108	0.0	0.0
10	12.0	Untreated	93	10.7	9.1
		Treated	91	0.0	0.0
11	35.0	Untreated	102	32.3	27.8
		Treated	96	0.0	0.0

The results of the second experiment are shown in Table 2. Eleven seed samples were treated and tested to ascertain whether the success of the first experiment could be repeated with other seed lots. Neither seedling evaluations nor field maturity tests detected any infected plants, whereas untreated seed gave rise to infected seedlings and heads in percentages slightly lower than the results of the embryo tests.

DISCUSSION

The method for seed therapy described above appears to disinfect barley seed without impairing seed germination, and eliminates any seed handling between the phases of treatment. The procedure appears to be practical enough for general use and, where uninfected barley seed is insufficient for planting, could be valuable in preventing loose smut epiphytotics. The drying schedule used, based on 8-hour periods, probably could be completed in one 24-hour operation. Further, warm air would accelerate drying considerably.

The results suggest that the seedling test provides a reliable evaluation of treatments of barley seed infected with Ustilago nuda, and generally shows a percentage of infection somewhat lower than that of the embryo test but higher than that of field maturity evaluations.

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NORTH DAKOTA AGRICULTURAL EXPERIMENT STATION, FARGO

METHYL BROMIDE FUMIGATION OF HETERODERA GLYCINES IN NORTH CAROLINA[1]

J. N. Sasser and Grover Uzzell, Jr.[2]

Summary

Eradication of the soybean cyst nematode with methyl bromide in an airtight fumigation chamber or under a polyethylene cover required the following combinations of temperature, exposure period, and dosage levels per 1000 cubic feet: 50° F, 2 hours, 16 pounds; 50°, 4, 8; 60°, 2, 8; 70°, 2, 8; 70°, 4, 4; 80°, 2, 8; 80°, 4, 4,; 90°, 4, 2. Dosages required under cover for corresponding temperature, exposure period and dosage level combinations were comparable to those required in the chamber. There was a close correlation between the Baermann funnel and bioassay methods for determining lethal dosages.

INTRODUCTION

Soon after the discovery of the soybean cyst nematode, Heterodera glycines Ichinohe, in North Carolina State authorities imposed a quarantine on the movement of soil, plant parts, machines and many other materials that might spread the nematode. With the finding of the nematode in Tennessee and Missouri, a Federal Quarantine containing provisions parallel to the North Carolina State Quarantine was established and became effective July 26, 1957. The establishment of these quarantines brought about the problem of setting up disinfesting procedures by cleaning with water, compressed air, steam, or by fumigation whereby regulated materials could be moved through normal channels.

During the fall of 1959 fumigation tests were conducted to determine lethal dosage levels of methyl bromide for the soybean cyst nematode at various temperatures and exposure periods. The tests were carried out in two ways: 1) in a fumigation chamber with controlled temperatures, and 2) under a polyethylene cover with no temperature control. The purpose of this paper is to report briefly the results of these tests.

CHAMBER FUMIGATION

The 100-cubic foot fumigation chamber was equipped with a metering cylinder and a thermostatically controlled heater (Fig. 1). The chamber temperature could be controlled so as not to drop below the desired temperature or exceed it by 4° F. A fumiscope[3] was used to measure the methyl bromide concentration in the chamber throughout the exposure period to assure the proper concentration of methyl bromide during treatment. A fan was run for 20 minutes at the beginning of treatments to circulate the gas in the chamber. The fan was used at the end of the treatments to expel the gas.

Forty-seven separate tests were made with dosages ranging from 1 to 16 pounds per 1000 cubic feet. Exposure periods of 1, 2, 3 and 4 hours at temperatures of 50°, 60°, 70°, 80° and 90° F were used. Temperatures were controlled to within 4° F above the desired temperature.

Heavily infested soil was used as test material. Approximately 1 tablespoonful of this soil was placed in each of five pairs (designated as A and B) of small open cardboard boxes and placed on stands inside the chamber. These stands were 2 feet high and were located at each end and in the middle of the chamber. Two samples (pairs designated as A and B) comparable to the five pairs that were placed in the chamber were held as controls for each test. After each test, samples designated A were assayed for viable larvae by the Baermann funnel method, allowing the cysts to stay in the funnels for 10 days. Temperatures of approximately 80° F were maintained. Larval counts were made in syracuse watch glasses with the aid of a dissecting microscope. Samples designated B were bioassayed by placing the cyst material in 4-

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[1] Cooperative tests by North Carolina Agricultural Experiment Station, North Carolina State Department of Agriculture, and Plant Pest Control Division, Agricultural Research Service, United States Department of Agriculture.

[2] Associate Plant Pathologist (Nematode Diseases), North Carolina Agricultural Experiment Station, North Carolina State College, and PPC Inspector, Plant Pest Control Division, Methods Improvement Operations, Agricultural Research Service, United States Department of Agriculture, respectively.

[3] Fumiscope Model E, Robert K. Hassler Co., P. O. Box 177, Altadena, California.

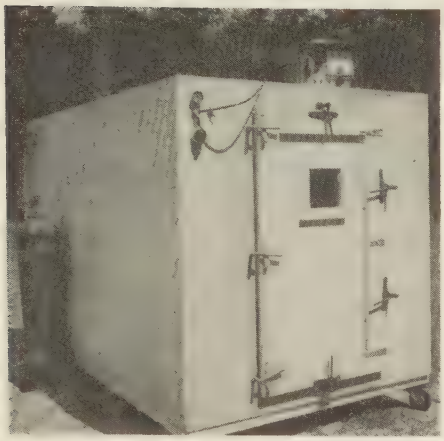

FIGURE 1. The 100-cubic foot fumigation chamber used in evaluating the efficacy of methyl bromide as an eradicant of the soybean cyst nematode.

FIGURE 2. The 375-cubic foot plastic covered frame used in evaluating efficacy of methyl bromide as an eradicant of the soybean cyst nematode.

inch pots of sterile soil and allowing susceptible soybeans to grow for 6 weeks prior to root examination for presence of cysts. Bioassays were made only for those samples treated at 70° and 80° F.

FUMIGATION UNDER COVER

A black polyethylene cover supported by a light metal frame was used in these tests (Fig. 2). By using a rectangular shaped metal frame to support the cover, it was possible to determine accurately the area to be fumigated. An enclosed volume of 375 cubic feet was used in these studies. No temperature control was used. An exhaust fan under the cover was run throughout the exposure period for the purpose of circulating the methyl bromide about the area. The fumiscope was again used to record the concentration of methyl bromide during treatment.

Infested material used for these tests was comparable to that used in the chamber tests and was placed in five small open cardboard boxes on stands dispersed at various heights under the cover. The stands on which the samples were placed ranged in height from ground level to 4 feet. For each test, two samples were held as controls.

A total of 10 tests were conducted with dosages ranging from 4 to 16 pounds per 1000 cubic feet. Exposure periods consisted of 2 and 4 hours. Assays were made using the Baermann funnel method as previously described. The bioassay method was not employed for those samples treated under cover.

EXPERIMENTAL RESULTS

Baermann Funnel Assays: Table 1 gives the results of the 47 combinations of temperature, exposure period and dosage which were evaluated for eradication of the soybean cyst nematode, as well as the corresponding number of larvae recovered from the untreated controls. Dosage appeared to have the greatest effect, although effectiveness of the chemical was also directly correlated with increases in temperature and exposure period. Lethal dosages required under the cover (Table 2) were closely correlated with those in the chamber for the approximate corresponding temperatures and exposure periods. A series of minimum dosage levels for the various temperature-exposure period combinations is given in Table 3.

Bioassay: Table 4 gives the results of those samples which were bioassayed in the greenhouse using susceptible soybeans as the indicator crop. Temperature, exposure period and dosage level combinations shown in Table 1, which were lethal to the nematode according to the Baermann funnel assay, were also lethal according to the bioassay tests. The check plants were severely infected, while plants which had grown in soil infested with treated cysts showed varying degrees of infection depending on the dosage.

Table 1. Efficacy of methyl bromide in a closed chamber at various temperatures, exposure periods and dosage levels as an eradicant for the soybean cyst nematode in soil.

Temperature (° F)	Exposure period (hours)	Dosage/1000 cubic feet (in pounds)	Average number of larvae recovered	
			Treated soil[a]	Non-treated soil[b]
50	1	8	765	765
	1	16	32	950
	2	8	115	790
	2	16	0	915
	3	4	602.0	1265
	3	8	0	1210
	3	16	0.4	850
	4	4	31	805
	4	8	0	1505
	4	16	0	1225
60	1	8	324	1320
	1	16	0.2	670
	2	8	0	500
	2	16	0.2	710
	3	4	2	660
	3	8	0	635
	3	16	0	2410
	4	4	10.0	1105
	4	8	0.2	1685
	4	16	0	1845
70	2	2	432	550
	2	4	15	295
	2	8	0	285
	2	16	0	575
	4	2	20	655
	4	4	0	365
	4	8	0	515
	4	16	0	900
80	2	2	60	233
	2	4	0	393
	2	8	0	300
	2	16	0	455
	4	2	0.4	385
	4	4	0	380
	4	8	0	920
	4	16	0	480
90	1	1	360	455
	1	2	306	815
	1	4	154	895
	1	8	0.4	585
	1	16	0	530
	2	1	396	685
	2	2	118	1265
	3	1	200	1045
	3	2	1.4	710
	4	1	140	1040
	4	2	0	905

[a]Average of five replications.
[b]Average of two replications.

Table 2. Efficacy of methyl bromide under a sealed cover at various temperatures, exposure periods and dosage levels, as an eradicant for the soybean cyst nematode.

Temperature (° F)	Exposure period (hours)	Dosage/1000 cubic feet (in pounds)	Average number of larvae recovered	
			Treated soil[a]	Non-treated soil[b]
50-60	2	8	1.0	1115
	2	16	0	1345
	4	4	0.2	2465
	4	8	0	915
	4	16	0	890
60-72	2	8	0	2350
	2	16	0	4925
	4	4	0	870
	4	8	0.8	3785
	4	16	0	4100

[a]Average of five replications.
[b]Average of two replications.

Table 3. Minimum dosages of methyl bromide per 1000 cubic feet required to eradicate the soybean cyst nematode at various temperatures and exposure periods. (Data taken from Tables 1 and 2).

Temperature (° F)	Exposure period	
	2 hours	4 hours
Chamber	Dosage in pounds	
50	16	8
60	8	-
70	8	4
80	8	4
90	-	2
Under Cover		
50-60	16	8
60-72	8	4

DISCUSSION AND CONCLUSIONS

These tests conducted under controlled conditions provide data on the required dosage levels of methyl bromide at various temperatures and exposure periods for the eradication of the soybean cyst nematode, under conditions approaching those comparable to cysts in soil adhering to farm machinery, crates, baskets and farm produce. Although methyl bromide has been evaluated for control of the soybean cyst nematode in soil (1), and as a space fumigant against the golden nematode of potatoes adhering to burlap bags and machinery (3), there are no reports in the literature relative to the effectiveness of methyl bromide applied as a space fumigant to control the soybean cyst nematode under similar conditions. Lear and Mai (3) found that 23 pounds of methyl bromide per 1000 cubic feet for 16 hours were required to eradicate the golden nematode of potatoes, and that 46 pounds per 1000 cubic feet for 2 hours was almost equally effective. This agrees with tests reported herein, in that initial dosage appeared to be more important than temperature or exposure period. These workers, however, reported that treatments were equally effective at temperatures between 50° and 80° F, which was not the case with the soybean cyst nematode. Temperature increases resulted in a much greater kill for the various exposure period-dosage combinations. A probable explanation for this may be the fact that inoculum used in tests with the soybean cyst nematode had been kept moist prior to the test and were probably easier killed than dried cysts. Desiccation, however, greatly affects the survival of the soybean cyst nematode and tests were not included using dried cyst material.

The close correlation between the results of the Baermann funnel assay and the bioassay indicates that the funnel method probably would be just as reliable as the bioassay method which

Table 4. Results of bioassay of soybean cyst nematode infested soil after treatment with methyl bromide at various temperature, exposure period and dosage combinations, on Lee Variety soybeans.

Temperature (° F)	Exposure period (hours)	Dosage/1000 cubic feet (in pounds)	Roots infected[a]	
			Treated	Check
70	2	2	+	+
	2	4	+	+
	2	8	-	+
	2	16	-	+
	4	2	+	+
	4	4	-	+
	4	8	-	+
	4	16	-	+
80	2	2	+	+
	2	4	+[b]	+
	2	8	-	+
	2	16	-	+
	4	2	+	+
	4	4	-	+
	4	8	-	+
	4	16	-	+

[a]Plus sign indicates that cysts were observed on the roots. Minus sign indicates that no infection (cysts) was observed.

[b]This was the only case where the results of the bioassay differed from those obtained in the Baermann funnels.

takes much longer. Since hatching of larvae from cysts does not require a hatching factor such as that found in potato root leachings in the case of the golden nematode, the funnel method is probably sufficient for tests involving the soybean cyst nematode. Feldmesser and Fassuliotis (2), however, have reported inhibition of emergence in cysts of the golden nematode treated with methyl bromide. In such cases of temporary inhibition of hatching, the bioassay method would be more reliable.

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NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION, RALEIGH AND PLANT PEST CONTROL DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

RECENT DEVELOPMENTS IN THE CONTROL OF STING NEMATODE, BELONOLAIMUS LONGICAUDATUS, ON PEANUTS WITH 1,2-DIBROMO-3-CHLOROPROPANE AND EN 18133[1]

J. N. Sasser, W. E. Cooper and T. G. Bowery

INTRODUCTION

The susceptibility of crops commonly grown in rotation with peanuts to sting nematode, Belonolaimus longicaudatus Rau, makes control by rotation impracticable. Corn, soybeans, and cotton -- crops most often grown in rotation with peanuts -- are all highly susceptible to sting nematode attack and, when grown, not only yield poorly but build up the nematode population, making almost certain a poor yield and quality of peanuts grown in these fields the following year. Chemical control, on the other hand, has proved highly successful (2, 3, 4, 6).

In 1958 investigations were conducted in North Carolina to determine effective rates and relative efficacy of preplant and postplant applications of 1,2-dibromo-3-chloropropane (DBCP) on sting nematode control, yield and quality of peanuts, corn, soybeans and cotton (2). In 1959 emphasis was placed on control of sting nematode in peanuts only. A preliminary report has been given (1).

MATERIALS AND METHODS

Location and Soil: The field selected for this experiment was on the Barkeley Estate about 1 mile northeast of Severn, North Carolina. It had a history of poor yields of all crops for several years. Soybeans and corn grown in 1958 and 1959, respectively, were severely affected by the sting nematode disease. The soil, a Norfolk loamy fine sand, was relatively uniform and was heavily infested with the sting nematode with very few other plant-parasitic forms present.

Soil Preparation: The debris of the preceding corn crop was buried during March by turning the soil. The field was disced and harrowed just prior to initiating the study. To reduce damage from soil inhabiting insects, 1 1/2 pounds (active)/acre of aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a,-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene) were applied just prior to discing.

Plot Design and Treatments: The experimental design was a randomized block with a single row (3 feet x 40 feet) split plots. The whole plots represented the date of treatment and the split plots materials, sources and/or rates.

Tests were designed to compare commercial sources, formulations and rate of applications of DBCP as preplanting, at planting and postplanting (3 and 6 weeks) treatments for sting nematode control. Also included were split applications with one-half of the chemical applied as a preplanting and the other half applied as a postplanting treatment at 3 and/or 6 weeks. A split application, one-half applied at planting and the other half 3 weeks after planting, was also included. A new nonfumigant nematocide, EN 18133, which gave promising results in a preliminary test in 1958, was included at three rates in the at planting series. Preplanting and at planting applications of granular (17.3%) DBCP were applied approximately 6 inches deep in the row with a modified Gandy distributor. For postplanting applications, a weighed quantity was applied by hand to an open furrow 4 to 5 inches deep about 6 inches to each side of the row and covered immediately following application.

The liquid formulations were applied with a gravity flow soil fumigant applicator to the middle of the row at a depth of approximately 6 inches.

Weighed quantities of 10% granular EN 18133 were distributed by hand in a 12- to 15-inch band on the row. It was then thoroughly mixed into the soil by rototillering twice.

DBCP and EN 18133 were applied broadcast at 3 gallons/acre and 16 pounds/acre, respectively. The DBCP (17.3% granular) was applied 2 weeks prior to planting and the EN 18133 (10% granular) was applied at time of planting by hand over plots 39 x 30 feet and mixed with the soil with a rototiller. Each treatment and an untreated check were replicated twice.

Cultural Practices: Following applications of the at planting treatments the N. C. 2 variety of peanuts was planted with a 2-row tractor-drawn planter. Subsequent cultural practices were those normally recommended by the North Carolina Agricultural Extension Service for peanut production in this area.

[1] The 1,2-dibromo-3-chloropropane was supplied by Shell Chemical Corporation and Dow Chemical Company, and EN 18133 (O,O-diethyl O-2-pyrazinyl phosphorothioate) by the American Cyanamid Company.

Determination of Nematode Populations: An appropriate number of soil plugs 6 to 8 inches deep were taken from the root zone of representative plots with a 1-inch diameter soil sampling tube on May 5, June 18, and August 26, 1959. The nematodes were recovered from the soil by a combination of screens and modified Baermann funnel technique. Counts were made under a dissecting microscope (36X).

Harvest Operations: The peanuts were dug with a two-row tractor-mounted digger on November 20, 1959. Plants from individual plots were stacked on small stack poles until dry, December 3, when they were picked with a carding type Victory peanut picker. After individual plot yields were determined, December 6, the pods from the five replicates of each treatment were bulked, thoroughly mixed, and two 1-kilogram samples drawn, one for chemical residue analysis and one for grade analysis. Just prior to picking, a hay (leaves and stems) sample was taken from each DBCP treated plot and control plots. The hay samples from the five replicates of each treatment were pooled for chemical residue analyses. Total bromides were determined by the method of Shrader (5) as modified by R. W. Young, Department of Biochemistry, Virginia Polytechnic Institute, Blacksburg, Virginia.

EXPERIMENTAL RESULTS

Preplant Treatments: Table 1 summarizes the results obtained from the preplant treatments relative to quality, market price, yield and acre value. Six of the chemical treatments were superior to the untreated checks in percentage fancy sized pods (F. S. P.), and all chemical treatments were superior to the check in percentage extra large kernels (X. L. K.). Except for the 1.0 gallon per acre rate of Fumazone (DBCP) applied as a liquid, yields for all chemical treatments were significantly higher than the control. Acre values are shown as the product of price/cwt and yields.

Treatments Applied At Planting: Table 2 summarizes the results obtained from treatments applied at time of planting relative to quality, market price, yields and acre value. Seven of the chemical treatments were superior to the checks in percentage F. S. P. and all chemical treatments were superior to the checks in percentage X. L. K. Yields for all chemical treatments were significantly higher than the control.

Postplant Treatments: Table 3 summarizes the results obtained from postplant treatments relative to quality, market price, yield and acre value. Chemical treatments for the first postplant as well as the second postplant treatments were superior to the untreated controls in quality (percentage F. S. P. and percentage X. L. K.), price/cwt, yields per acre and acre values. In general, the second postplant treatments were more effective than the first.

Broadcast Applications: Table 4 summarizes the results obtained from broadcast applications of 1,2-dibromo-3-chloropropane and EN 18133 relative to quality, price/cwt, yield and acre value. Both chemical treatments were superior to the controls. The EN 18133 was more effective than Nemagon relative to quality, price/cwt, yield and acre value.

Nematode Control and Growth Index: Table 5 shows average nematodes per pint of soil for the various sampling dates as well as a growth index taken at the time of the last sampling for nematodes (August 26, 1959). In general, nematodes were highest in the controls for each sampling date. Varying degrees of control were obtained with the nematocides. Growth index in most cases was indirectly correlated with nematode control. A high nematode count was associated with a low growth index and vice versa.

Bromide Residue: Table 6 shows the total bromide content of shelled peanuts and peanut hay from plots treated with DBCP. The peanut hay analysis showed a much higher total bromide content than the shelled peanuts. The 1.5 gallon per acre applied as a liquid at planting showed the highest bromide residue in the shelled peanuts and peanut hay.

DISCUSSION AND CONCLUSIONS

The data presented demonstrate that control of the sting nematode results in increased quality, higher market price/cwt and increased yields. Since quality grade determines market price/cwt, improved quality in addition to increased yields resulted in substantial acre value increases: preplant, 76.50 to 309.18; at planting, 94.32 to 339.86; postplant, 71.34 to 238.49. Treatments applied at planting were as effective as preplant treatments. This should enable the grower to combine his operations so as to treat and plant at the same time. Preplant and at planting treatments were better than postplant applications. Postplant sidedress applications were more effective when combined with a preplant application, rather than when applied all at one time. Postplant applications applied all at one time, however, greatly increased acre

Table 1. Influence of fumigation for sting nematode control on peanut quality, market price, yield and value per acre. (Preplant treatments)

Treatment (gallons/acre)	F.S.P.[a] (%)	X.L.K.[b] (%)	Price/ cwt	Pounds/ acre	Acre value (in dollars)
DBCP (Nemagon) Gran. 1.0	35	52	11.02	2219**	244.53
Liq. 1.0	19	48	10.12	2115**	214.03
Liq. 1.5	28	49	10.00	2056**	205.60
DBCP (Fumazone) Gran. 1.0	37	55	11.17	2768**	309.18
Liq. 1.0	16	32	9.89	1629	161.10
Liq. 1.5	28	44	10.20	2192**	223.58
Nemagon Gran. 0.5 (plus 0.5 gallon sidedress 3 weeks after planting)	28	51	10.58	2605**	275.60
Nemagon Gran. 0.5 (plus 0.5 gallon sidedress 6 weeks after planting)	38	51	11.23	2628**	295.12
Untreated check	19	31	6.00	1275	76.50
L.S.D. at 5%				388	
1%				513	

[a]Fancy sized pods.
[b]Extra large kernels.

Table 2. Influence of soil treatments for sting nematode control on peanut quality, market price and value per acre. (Treatments applied at planting)

Treatment	F.S.P.[a] (%)	X.L.K.[b] (%)	Price/ cwt	Pounds/ acre	Acre value (in dollars)
DBCP (Nemagon) Liq. 0.5 gallons/acre	16	35	6.00	1656**	99.36
Liq. 1.0	18	38	9.67	1865**	180.34
Liq. 1.5	37	50	10.48	2196**	230.14
Gran. 1.0	58	51	11.09	2686**	297.88
EN 18133 4 pounds/acre	36	44	9.97	1947**	194.11
8	41	54	11.46	2451**	280.88
16	43	52	11.28	3013**	339.86
Nemagon Liq. 0.5 gallon/acre (followed by 0.5 gallon sidedress 3 weeks after planting)	44	50	10.15	1915**	194.37
Untreated check	16	28	9.00	1048	94.32
L.S.D. at 5%				388	
1%				513	

[a]Fancy sized pods.
[b]Extra large kernels.

Table 3. Influence of fumigation for sting nematode control on peanut quality, market price and value per acre. (Postplant treatments)

Treatment (gallons/acre)	F.S.P.[a] (%)	X.L.K.[b] (%)	Price/ cwt	Pounds/ acre	Acre value (in dollars)
1st Postplant Treatment (3 weeks after seeding)					
DBCP (Nemagon) 1.0	36	34	7.85	1679**	131.80
1.5	42	47	10.35	1956**	202.44
Untreated check	12	21	6.00	1057	63.42
2nd Postplant Treatment (6 weeks after seeding)					
DBCP (Nemagon) 1.0	34	39	9.81	1888**	185.21
1.5	51	51	10.88	2192**	238.49
Untreated check	19	26	6.00	1189	71.34
L.S.D. at 5%				388	
1%				513	

[a]Fancy sized pods.
[b]Extra large kernels.

Table 4. Influence of soil treatments for sting nematode control on peanut quality, market price and value per acre. (Broadcast applications)

Treatment	F.S.P.[a] (%)	X.L.K.[b] (%)	Price/ cwt	Pounds/ acre	Acre value (in dollars)
DBCP (Nemagon) 3.0 gallons/acre	36	44	9.51	1856	176.50
EN 18133 16 pounds/acre	49	57	11.43	2703	308.95
Untreated check	30	33	6.00	1291	77.46

[a]Fancy sized pods.
[b]Extra large kernels.

Table 5. The influence of nematocide treatments on sting nematode populations and growth of the peanut plants.

Treatment	Average sting nematodes/pint soil: May 5[a] 1959	June 18[b] 1959	August 26[e] 1959	Growth index[i] August 26 1959
Preplant Treatments				
DBCP (Nemagon) Gran. 1 gallon	25	5	35	3
Liq. 1	20	10	70	2.9
Liq. 1 1/2	30	15	45	3
DBCP (Fumazone) Gran. 1	13	0	45	3
Liq. 1	35	40	110	2.8
Liq. 1 1/2	20	5	70	3
DBCP (Nemagon) Gran. 0.5 followed by 0.5, 3 weeks after planting	55	20	60	3
DBCP (Nemagon) Gran. 0.5 followed by 0.5, 6 weeks after planting	15	10	55	2.9
Check	70	215	170	2.4
Treatments Applied At Planting				
DBCP (Nemagon) Liq. 1/2 gallon		20[c]	85[f]	2.7
Liq. 1		250	105	2.3
Liq. 1 1/2		15	75	2.9
Gran. 1		5	50	3
EN 18133, 40 pounds 10% Gran.		115	115	2.8
80		90	35	3
160		80	30	3
Check	135	205	185	1.5
DBCP (Nemagon) Liq. 1/2 gallon followed 3 weeks later with 0.5 gallon		35	65	2.6
1st Postplant (3 weeks after seeding)				
DBCP (Nemagon) 1 gallon		110[d]	80[g]	2.4
1 1/2		185	100	2.3
Check		125	160	1.7
2nd Postplant (6 weeks after seeding)				
DBCP (Nemagon) 1 gallon			75[h]	2.1
1 1/2			50	2.2
Check			155	1.7

[a], indicates plots were sampled 3 weeks after treatment; [b], indicates plots were sampled 8 weeks after treatment; [c], 6 weeks after treatment; [d], 3 weeks after treatment, [e], 19 weeks after treatment; [f], 17 weeks after treatment; [g], 13 weeks after treatment; [h], 10 weeks after treatment; [i], general growth and vigor of plants in the various plots were rated using a scale of 0-3, with 3 indicating excellent growth. Index shown represents the average of five replications.

Table 6. Total bromide content of shelled peanuts and peanut hay from plots treated with DBCP.

Treatment	Rate (gallons/acre)	Total bromide (ppm)[a]	
		Shelled peanuts	Peanut hay
Preplant-Broadcast	3	0	69
Preplant-Granular	1.0 in row	0	138
Preplant-Liquid	1.0 in row	17	69
Preplant-Liquid	1.5 in row	17	103
Preplant-Granular	0.5 in row followed by 0.5 3 weeks after planting	17	155
Preplant-Granular	0.5 in row followed by 0.5 6 weeks after planting	17	51
At Planting-Liquid	0.5 in row	0	34
At Planting-Liquid	1.0 in row	17	69
At Planting-Liquid	1.5 in row	34	242
At Planting-Granular	1.0 in row	0	207
At Planting-Liquid	0.5 followed by 0.5 3 weeks later	0	103
Postplant-Granular	1.0, 3 weeks after planting	17	121
Postplant-Granular	1.5, 3 weeks after planting	0	190
Postplant-Granular	1.0 in row 6 weeks after planting	17	69
Postplant-Granular	1.5 in row 6 weeks after planting	17	103
Untreated check		0	64

[a]Each value corrected for check.

value over the untreated controls and should prove valuable in fields where the infestation is discovered after the crop is growing. Why the later postplant application (6 weeks) was more effective than the earlier one (3 weeks) is not known.

Broadcast applications with EN 18133 show considerable promise. This nonfumigant nematocide lends itself to this type operation while 1,2-dibromo-3-chloropropane, a volatile material is more effective when injected or placed at least 6 inches deep.

Total bromide content of the shelled peanuts was low, especially at the 0.5 and 1.0 gallon per acre rates. The peanut hay, however, should not be fed to milk cows or to animals being finished for slaughter. Further studies should be made before using hay from treated fields as feed.

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DISEASES CAUSED BY TOBACCO RINGSPOT VIRUS IN THE LOWER RIO GRANDE VALLEY OF TEXAS

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Summary

Symptoms of tobacco ringspot virus (TRSV) on various plant species in the greenhouse during the winter of 1959-60 were compared with symptoms on mature inoculated plants in the field during the period February through June 1960. In the greenhouse TRSV incited symptoms in the following suscepts: cantaloupe, cucumber, watermelon, squash, bean, cowpea, lima bean, soybean, crotalaria, pea, guar, eggplant, petunia, tobacco, zinnia, sunflower, snapdragon, and sesame. On the basis of symptom similarities, serological reactions, and results from indexing trials, it is concluded that the virus causing eggplant yellows, watermelon pimples, cantaloupe mosaic, bean top-necrosis, and soybean bud-blight observed in the Lower Rio Grande Valley is a yellow strain of TRSV.

Although an insect vector of tobacco ringspot virus (TRSV) is unknown, the virus is widely distributed in a number of different hosts in Texas, particularly in the Lower Rio Grande Valley. Several vegetables are seriously affected and virus spread is rapid. Infections in different species in the Rio Grande Valley are as common in the spring as in the fall.

Workers in Texas and elsewhere reported TRSV as the cause of important plant diseases in different species. In the present studies the virus was isolated from naturally infected eggplant, cantaloupe, watermelon, squash, bean, crotalaria, soybean, and white horse nettle (Solanum elaeagnifolium) in the Rio Grande Valley. A wide range of test plants were index-inoculated with the virus from these sources.

This article lists the species experimentally infected by TRSV and describes the symptoms manifested in different test plants by the virus isolated from different host plants in the Rio Grande Valley. A preliminary report has been made (8).

METHODS AND MATERIALS

Symptom and host-range studies were carried out in the greenhouse during the winter of 1959-60 at temperatures of 65° to 80° F. Test plants were grown in 4-inch plastic pots except when certain inoculated ones were transplanted to the ground to observe symptoms longer in older plants.

Inoculations were made from virus-juice extracts by the leaf-rubbing method, using cloth swabs and carborundum powder (320) as abrasive.

To observe symptoms further in older plants, the virus was isolated from the rind of watermelon showing "pimples," eggplant leaves showing "yellows," and bean plants showing symptoms of top necrosis. Inoculations with the virus from these sources were made in February to plants of watermelon (Citrullus vulgaris var. Black Diamond), cantaloupe (Cucumis melo vars. PMR 6 and Rio Gold), cucumber (Cucumis sativus var. Palomar), squash (Cucurbita pepo var. Early Straight Neck), eggplant (Solanum melongena var. esculentum (Black Beauty)), and tobacco (Nicotiana tabacum var. Burley) growing in 3-inch peat pots in the greenhouse and transferred to field plots in March 1960. The following test plants were direct-seeded among the inoculated plants to study natural spread of the virus; crotalaria (Crotalaria intermedia), sesame (Sesamum indicum), cowpea (Vigna sinensis var. Blackeye), guar (Cyamopsis tetragonoloba), lima bean (Phaseolus lunatus var. Thorogreen), bean (Phaseolus vulgaris var. Black Valentine), petunia (Petunia violacea), soybean (Glycine max var. Harosoy), and sunflower (Helianthus annuus var. Mammoth Russian). The plants were grown to maturity (June) and symptoms were compared with those on inoculated plants in the greenhouse.

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RESULTS AND DISCUSSION

The symptoms manifested in different test plants after inoculation with the TRSV isolates from different hosts in the Rio Grande Valley are listed in Table 1.

Symptoms on muskmelon, cucumber, watermelon, squash, tobacco, bean, and pea inoculated with the virus from different host sources in Texas were identical with those on similar test plants as described by Pound (9) in Wisconsin. He isolated a mosaic virus from watermelon and cantaloupe which produced identical symptoms in different test plants and concluded that the virus was a yellow strain of TRSV. Sinclair and Walker (12) showed TRSV to be widespread in cucumbers in central Wisconsin during 1953 through 1955. Shepherd and Struble (11) in Oklahoma and Rosberg (10) in Texas correlated a "pimples" disease of watermelon fruits with infection by a yellow strain of TRSV which incited similar symptoms in the test plants used by Pound.

Ivanoff (6) in Texas transferred the eggplant "yellows" virus from eggplant to cucumber and induced yellows symptoms in eggplant by inoculating with a virus causing "mosaic" symptoms in cantaloupe. Later, Valleau (14) in Kentucky inoculated tobacco with juice extracted from yellows eggplants obtained from Texas and concluded that eggplant yellows was caused by a yellow strain of TRSV. He stated that TRSV is probably more important in causing mosaic of cucumber in Kentucky than are the typical cucumber mosaic viruses (13).

Allington (1) in Illinois and Athow and Bancroft (2) in Indiana have reported a seed-borne bud blight of soybean which cross-protection tests in tobacco demonstrated to be caused by TRSV. Soybean bud-blight isolated from infected seed of the Harosoy variety supplied by K. L. Athow have resembled other Rio Grande Valley TRSV isolates in their reactions on various test plants in the present studies.

In 1958 an experimental planting of guar in the Lower Rio Grande Valley showed symptoms similar to those of top necrosis described by Cooper (5) to be caused by TRSV in Oklahoma. In Texas, guar inoculated with virus isolates from naturally infected eggplant (yellows), watermelon (pimples), soybean (bud-blight), cantaloupe (mosaic), and bean (top necrosis) (8) produced similar symptoms.

Chester and Cooper (4), in studies with a lethal virus of guar, failed to infect Nicotiana glutinosa and, on the basis of symptoms produced in N. tabacum and petunia, felt that the guar virus differed from TRSV. Cooper (5) transferred the guar virus from symptomless inoculated N. glutinosa plants to cowpea, but failed to obtain symptoms in pea or crotalaria. He considered the top-necrosis virus of guar a distinct strain of TRSV for which he proposed the name Annulus tabaci Holmes var. cyamopsidis (5).

Rosberg (10) did not obtain symptoms in petunia with the TRSV from pimples watermelon in Texas, whereas Shepherd and Struble (11) infected petunia with a virus from pimples watermelons in Oklahoma.

Cheo and Zaumeyer (3) reported from Virginia and Delaware a strain of TRSV which caused top necrosis in bean and typical symptoms of TRSV infections in tobacco and cowpea. LeBeau (7) reported a virus-induced top necrosis in beans in Mississippi and, on the basis of the physical properties of the virus and symptoms expressed in different test plants, concluded that the virus was probably related to the soybean bud-blight virus and the guar top-necrosis virus.

Like crotalaria plants inoculated with TRSV experimental plants of crotalaria grown in the field at Weslaco are usually stunted, chlorotic, and proliferated.

The Texas isolate of TRSV incited local lesions on inoculated primary leaves of cowpea and lima bean in these experiments. Lima bean showed a hypersensitive reaction. Lesions on inoculated leaves were necrotic and affected leaves abscised. The virus was not recovered in subsequent growth nor was any protective action discernible after challenge inoculations to trifoliate leaves. The virus became systemic and usually lethal in cowpea seedlings and was isolated from trifoliate leaves of inoculated plants. Plants that survived a primary inoculation usually succumbed after a second inoculation. The virus was not generally recovered from inoculated watermelon and sunflower seedlings; occasionally the virus was transferred from infected watermelon to cucumber. Shepherd and Struble (11) demonstrated a virus inhibitor in juice extracts from watermelon.

Serological tests conducted in cooperation with Howard Scott, Plant Virology Laboratory, Beltsville, Maryland, with the virus isolated from cantaloupe, bean, watermelon, eggplant, and soybean showed the virus is related to Steere's ringspot virus (AC-174, American Type Culture). Antiserum for these tests was obtained from Microbiological Associates, Bethesda, Maryland.

Table 1. Symptoms produced on selected species and the degree of severity of a strain of TRSV from different hosts.

Test species	Local symptoms	Systemic symptoms	Severity rating[a] virus source				
			eggplant	watermelon	cantaloupe	bean	soybean
Cantaloupe	chlorotic (pinhead-size) stippling, ring spots	coarse yellow mottle, necrosis of crown leaves, stunting, eventually masked	2	2	1	1	1
Cucumber	chlorotic rings	coarse yellow mottle, often masked	2	3	3	3	3
Watermelon	zonate chlorotic stipple, sometimes necrotic	severe stunting of terminals, distortion of leaves, necrosis, intumescence on fruit, eventually masked	1	1	1	1	1
Squash	chlorotic ringspots	stunting, coarse yellow mottle, eventually masked	1	1	1	1	1
Bean	necrotic lesions on inoc. leaves, epinasty	necrosis of shoot, elongated cankers on petioles and stem, vein necrosis, mild mottling	2	2	2	2	2
Cowpea	reddish necrotic lesions	severe shoot necrosis, usually lethal, necrotic stem lesions, vein necrosis	2	2	3	3	3
Soybean	chlorotic lesions on inoc. leaves, epinasty	bronzing of foliage, stunting, abscission of leaves, prominent pubescence, brittleness of new growth	2	2	2	2	2
Lima bean	concentric necrotic lesions on inoc. leaves, vein necrosis, hypersensitive	none	2	2	2	2	2
Pea (Pisum sativum)	chlorotic stipple on inoc. leaves	wilting, chlorosis, lethal	3	3	3	3	3
Crotalaria	chlorotic stipple	chlorosis, stunting, often lethal in seedlings, abscission of leaves	2	2	2	2	2
Guar	necrotic lesions on inoc. leaves	systemic necrosis, lethal	3	3	3	3	3
Eggplant	none	chlorotic blotches on foliage becoming completely chlorotic, typical yellows symptoms	3	3	3	3	3
Petunia	small ringspots on inoc. leaves	severe chlorosis, often recovery in young plants	2	2	2	2	2
White horse nettle	none	symptoms same as in eggplant but milder, not always manifested in young plants	1	1	1	1	1
Tobacco	necrotic lesions, etched rings	ringspotting, jagged oak-leaf to lightening-like pattern, general diffuse chlorosis, recovery	1	3	1	1	1
Nicotiana repanda	necrotic lesions on inoc. leaves	none, virus not recovered	-	-	-	-	-
Nicotiana glutinosa	none	very mild chlorosis, virus recovered to cowpea	-	-	-	-	-
Tomato (Lycopersicon esculentum)	none	none	-	-	-	-	-
Pepper (Capsicum frutescens var. Yolo Wonder)	none	none	-	-	-	-	-
Zinnia (Zinnia elegans)	none	mild chlorotic mottle, recovery	1	1	1	1	1
Sunflower	mild ring spotting	mild mosaic, recovery, virus not recovered	1	1	1	1	1
Head lettuce (Lactuca sativa var. capitata)	none	none	-	-	-	-	-
Snapdragon (Antirrhinum majus)	chlorotic lesions becoming necrotic	severe chlorosis and brittleness	3	3	3	3	3
Sesame	necrotic (frogeye) lesions	systemic necrosis, lethal	3	3	3	3	3

[a]Numerical rating based on severity of symptoms expressed, period of incubation, and relative ease of mechanical trans-

Except for apparently longer incubation periods in certain cases, differences in intensity of symptom expression, and greater numbers of local lesions in certain test plants when inoculated with the virus from different hosts, the virus causing eggplant yellows, watermelon pimples, cantaloupe mosaic, bean top-necrosis, and soybean bud-blight in the Lower Rio Grande Valley appears to be a yellow strain of TRSV.

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OCCURRENCE OF BACTERIAL BLIGHT OF SUGAR BEETS IN MARYLAND

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Abstract

Bacterial blight occurred on sugar beet plants in a seed plot at the Plant Industry Station, Beltsville, Maryland in 1959. Cultural studies and inoculation tests indicate that the causal organism is Pseudomonas aptata (N. A. Brown & Jamieson) F. L. Stev., listed also by earlier writers as pathogenic on nasturtium, bean, eggplant, pepper, and lettuce. Spinach, New Zealand spinach, sesame, and globe-amaranth, as well as the previously reported hosts, were infected experimentally with the organism isolated from sugar beets.

INTRODUCTION

Bacterial blight, or black streak, of sugar beets, caused by Pseudomonas aptata (N. A. Brown & Jamieson) F. L. Stev., has been reported in Utah (4), California, Washington, and Oregon (1, 5). A leaf spot disease of nasturtium (Tropaeolum majus), caused by the same pathogen, has been reported from Maine (6), Minnesota (7), Mississippi (2), New Jersey (8), Pennsylvania (3), Texas (8), and Virginia (4).

OCCURRENCE OF THE DISEASE

In June 1959, symptoms of bacterial blight were observed on 11 of approximately 1000 sugar beet plants in a seed plot at the Plant Industry Station, Beltsville, Maryland. The upper stems and inflorescences were streaked dark brown, and necrotic blotches occurred on the leaves. Some terminal shoots had died prematurely. Microscopic examination of portions of diseased stems and flowers revealed masses of motile, rod-shaped bacteria oozing from the tissues. No symptoms of the disease were observed on beets in other plots in the vicinity.

Seed was harvested from the blighted plants in August. Five months later, 100 seed balls from each of eight blighted plants were planted in sterilized sand in the greenhouse. Of the 640 seedlings that emerged, 10.4% showed black lesions surrounded by a yellow halo on the cotyledons. These lesions yielded motile, rod-shaped bacteria with cultural and pathogenic characteristics of Pseudomonas aptata. The symptoms closely resembled those attributed to P. aptata and confirm the report that the disease is transmitted through the seed balls (1).

THE CAUSAL ORGANISM

A motile, rod-shaped bacterium was consistently isolated from blighted plants. The bacteria measure 1.7 x 0.6μ, are Gram-negative, and have polar flagella. In certain media, such as Uschinsky's solution, the cultures produce a green fluorescence. Other morphological and cultural characters of the organism also conform with those ascribed to P. aptata (4).

Inoculation tests were made in the greenhouse to determine whether the host range of the bacterium conforms to that of P. aptata. Two methods of inoculation were employed: 1) stems and petioles were punctured with a fine needle pushed through a drop of bacterial suspension placed on the plant surface; and 2) leaves previously dusted with carborundum were swabbed with a bacterial suspension.

The isolates were highly pathogenic on sugar beet, causing necrosis of the petioles and leaf blades. On nasturtium they caused leaf spot symptoms similar to those attributed to P. aptata (4). The isolates were also pathogenic on the reported experimental hosts of P. aptata including bean (Phaseolus vulgaris), eggplant (Solanum melongena), and pepper (Capsicum frutescens) and were weakly pathogenic on lettuce (Lactuca sativa).

The following additional species were found to be hosts of the bacterium: spinach (Spinacia oleracea), New Zealand spinach (Tetragonia expansa), sesame (Sesamum indicum), and globe-amaranth (Gomphrena globosa). Symptoms on spinach and sesame included brown streaks on the stems and blighting of the leaves above the point of inoculation. Symptoms on New Zealand spinach included gray, necrotic lesions on the stems and blighting of the leaves. Young plants of globe-amaranth became severely wilted and died within 2 weeks after inoculation. On older plants of this species the organism caused rotting and collapse of the stem and blighting of

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leaves, immediately above the point of inoculation. The bacterium was reisolated from inoculated plants of each susceptible species and was identified as Pseudomonas aptata.

The organism was not pathogenic on the following species: Carthamus tinctorius, Chenopodium album, and Nicotiana tabacum.

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TEST OF FOUR FUNGICIDES FOR CONTROL OF CEDAR BLIGHT[1]

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Summary

Eastern redcedar seedlings (1-0, 2-0, and 3-0) in an eastern Nebraska nursery were treated with Puratized Agricultural Spray, Kromad, 1.3% cycloheximide, and 65% dodine to determine the effectiveness of these chemicals in controlling cedar blight.

Puratized Agricultural Spray gave superior blight control in 1-0 and 2-0 redcedar seedlings. Blight incidence in Kromad-treated plots was much lower than in the checks but much higher than in the Puratized Agricultural Spray-treated beds. Cycloheximide 1.3% at dosage of 1.6 ounces/10 gallons of water and 65% dodine at dosage of 0.2 pound/10 gallons of water were phytotoxic. Dodine at reduced dosage, 0.1 pound/10 gallons of water, was not toxic.

Blight incidence in the 3-0 seedlings in the experimental bed and in other beds throughout the nursery was very light. Treatments did not significantly influence amount of blight in the 3-0 seedlings.

Cedar blight (Phomopsis juniperovora Hahn) frequently causes severe losses of eastern redcedar (Juniperus virginiana) in Plains nurseries. Bordeaux mixture and Special Semesan have frequently been used but have not given satisfactory control. Recently several nurseries have used Puratized Agricultural Spray (phenyl mercuri triethanol ammonium lactate). However, Plains nurseries have not properly measured the effectiveness of this fungicide. The trial herein reported was made to test the efficacy of Puratized Agricultural Spray and three other comparatively new fungicides for control of cedar blight in a Plains forest tree nursery.

MATERIALS AND METHODS

Four fungicides were tested on three eastern redcedar beds (1-0, 2-0 and 3-0)[5] in an eastern Nebraska nursery. Each bed was 300 feet in length and contained five rows which were 8 inches apart. These beds had been fall-sown with seed obtained from natural stands along the Platte River. Seedling density was determined by sampling in each experimental bed in October 1959. The number of seedlings per square foot was: 41 for 1-0, 30 for 2-0, and 37 for 3-0. The 2-0 and 3-0 beds selected for the test had not been seriously affected by blight the previous season.

Experimental Design: A randomized block design was used. Each bed was divided into four blocks. Each block contained five plots separated by 7-foot untreated isolation plots.

Method of Application: The chemicals were applied to the plots with a high pressure Century sprayer at 200 to 300 p.s.i. The beds of 2-0 and 3-0 redcedar were treated May 14, May 26, June 5, July 3, July 30, August 8, August 17, August 30, and September 9, 1959. The 1-0 redcedar bed was first sprayed July 3 and thereafter on above scheduled dates.

Treatments:

Puratized Agricultural Spray (7.5% phenyl mercuri triethanol ammonium lactate)	1 1/2 pints/55 gallons water
Kromad (5% cadmium sebacate; 5% potassium chromate; 1% malachite green; 0.5% auramine; 16% thiram)	0.2 pound/10 gallons water
Dodine (Cyprex 65-W) (65% n-dodecylguanidine acetate)	0.1 pound/10 gallons water

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[5]1-0 = 1-year-old nontransplanted seedlings, 2-0 = 2-year-old nontransplanted seedlings, 3-0 = 3-year-old nontransplanted seedlings.

Dosage reduced (July 3)	0.1 pound/10 gallons water
Cycloheximide (Acti-dione RZ) (1.3% cycloheximide (beta [2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide); 75.0% pentachloronitrobenzene	1.6 ounces/10 gallons water
Dosage reduced (July 20)	0.11 ounce/10 gallons water
Check (not treated)	

Data Collection: The number of blighted seedlings in samples from all plots was determined in October. A wire was placed across the plots at randomly selected intervals; the first five plants on each side of the wire in each of the five rows constituted the sample of 50 plants.

In the 1-0 bed two samples were taken in each plot, and in the 2-0 and 3-0 beds one sample was taken per plot. In the 1-0, 2-0, and 3-0 plots data were collected on the number of blighted plants in each sample. In the 2-0 and 3-0 plots each blighted plant was given a disease severity rating. Top weight (green) of the seedlings in samples from the 2-0 and 3-0 plots was also determined.

The seedlings were not inoculated with cedar blight fungus before treatment, reliance being placed on natural infection. The first infections were evident on 2-0 redcedar in late May. Because of an unusually wet growing season, the causal fungus remained very active throughout the summer and early fall, except in 3-0 beds where the disease failed to become intense. Redcedars in four untreated 1-0 beds were so severely infected that eventually all seedlings in these beds were removed and destroyed by the nursery.

RESULTS

The fungicides significantly reduced cedar blight in the 1-0 and 2-0 stock, Puratized Agricultural Spray giving the best control (Table 1). However, in the 3-0 beds where the disease was not severe, chemical treatment did not significantly influence disease incidence.

Table 1. Incidence of cedar blight in eastern redcedar seedlings following various fungicidal treatments.

Treatment	Age of seedlings		
	1-0	2-0	3-0
	Average incidence of blight[a]		
Puratized Agricultural Spray	5.6	8.3	10.5
Kromad	8.6	32.3	5.3
Dodine 65%[b]	10.4	--	--
Check	17.1	42.0	7.0
L.S.D. 0.05	4.57	5.07	n.s.

[a]Each datum represents the average number of blighted plants among 50 plants examined in each replication.

[b]Chemical burn occurred in 2-0 and 3-0 material to the extent that disease readings could not be taken.

Dodine was phytotoxic on the 2-0 and 3-0 seedlings at the dosage of 0.2 pound/10 gallons of water. These seedlings recovered somewhat when the dosage was reduced to 0.1 pound/10 gallons of water. The 1-0 seedlings, which were treated with dodine only at the reduced rate, did not show toxic reactions. Cycloheximide caused such severe chemical burn that no readings could be taken. The dosage rate was reduced from 1.6 ounces to 0.11 ounce/10 gallons of water to alleviate phytotoxicity. However, toxicity was still severe at this lower rate.

A measure of the amount of blighted foliage on treated plants at the end of the growing season is given in Table 2. Puratized Agricultural Spray-treated 2-0 plants had much less blighted foliage than 2-0 plants treated with Kromad.

There were significant differences between treatments in top weight of the 2-0 material (Table 3). The Puratized Agricultural Spray-treated plants averaged nearly 2 1/2 times more weight than the checks. No significant weight difference existed in the 3-0 material.

DISCUSSION

Puratized Agricultural Spray gave good control of cedar blight in 1-0 and 2-0 redcedar beds. Caroselli (1) in New Jersey obtained good control of blight with another phenyl mercury

Table 2. Cedar blight incidence and severity in 2-0 and 3-0 seedlings treated for blight control. October 1959.

Treatment[b]	Incidence and severity[a]				
	0	1	2	3	4
	2-0 Seedlings				
Puratized Agricultural Spray	167	23	1	1	8
Kromad	71	46	13	5	65
Check	32	35	23	18	92
	3-0 Seedlings				
Puratized Agricultural Spray	158	34	5	2	1
Kromad	179	20	0	0	1
Check	172	26	2	0	0

[a]Blight severity of infected seedlings was rated according to percentage of blighted foliage: 0 - blight free, 1 - 1-25% blighted, 2 - 26-50% blighted, 3 - 51-75% blighted, 4 - 76-100% blighted.
[b]200 plants (50 x 4 replications) from each treatment were rated.

Table 3. Top weight (green) of 2-0 and 3-0 redcedar seedlings treated for blight control.

Treatment	Age of seedlings	
	2-0	3-0
	Weight in grams[a]	
Puratized Agricultural Spray	4.6	7.2
Kromad	2.7	6.5
Check	1.9	7.2
L.S.D. 0.05	.86	n.s.

[a]Each datum represents an average of the top weight of 200 seedlings.

compound, Merbam (phenyl mercury dimethyl dithio carbamate). He also obtained good control with Kromad; however, in this test blight incidence was high in Kromad-treated 2-0 seedlings.

In this experiment, cycloheximide (Acti-dione RZ) injured the foliage of 1-0, 2-0, and 3-0 eastern redcedar when treated at rates of 1.6 ounces (1200 ppm) and later at 0.11 ounce (82 ppm)/10 gallons water. This was not expected as WK-34, an early formulation which led to the development of Acti-dione RZ, was used at a rate of 1.2 ounces/10 gallons of water and did not injure redcedar foliage (1). Strong and Klomparens (2) had used 100 ppm Acti-dione (cycloheximide) applied in the spring on mature redcedar to prevent germination of the teliospores of the cedar gall without injury to the foliage. The Upjohn Company, manufacturers of Acti-dione RZ, in reply to our inquiry concerning the toxic action of this fungicide, stated that 12 ppm of Acti-dione RZ is currently recommended for control of cedar blight.

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NEBRASKA AGRICULTURAL EXPERIMENT STATION, LINCOLN AND ROCKY MOUNTAIN FOREST AND RANGE EXPERIMENT STATION, FOREST SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

HOST RANGE STUDY OF THE SPIRAL NEMATODE, HELICOTYLENCHUS MICROLOBUS[1]

Donald P. Taylor[2]

Abstract

Of 127 plant varieties tested, 94 were found to be hosts of the spiral nematode, Helicotylenchus microlobus. All varieties tested of field corn, oats, barley, rye, common and durum wheats, sugar beet, red clover, potato, and soybean (except the variety Hawkeye) were rated as hosts.

INTRODUCTION

Helicotylenchus microlobus Perry, 1959 was one of the most frequently observed spiral nematodes in soil samples collected in Minnesota from 1956-1959, occurring in 8% of 810 samples[3]. Although frequently collected, no information was available on the host range of this nematode.

MATERIALS AND METHODS

The original collection of H. microlobus used in this study was obtained from a soil sample collected from the crop sequence plot of the Department of Plant Pathology and Botany, Minnesota Agricultural Experiment Station, at Rosemount. A large soil sample was taken from an area of high infestation and processed for nematode recovery using standard techniques. Batches of 5000 individuals of H. microlobus were handpicked from the mixed nematode population obtained. These batches were poured over the root systems of Marglobe variety tomato plants maintained in a 75° F greenhouse in 8-inch clay pots. All or a portion of the soil from a pot was processed for nematode recovery when needed.

Seeds or rooted cuttings of plants to be tested were planted in autoclaved sand in steamed 2-inch clay pots, which were then placed within 6-inch clay pots containing autoclaved greenhouse soil in such a way that the top of the small inner pot was slightly higher than that of the outer pot. Two pots of each test plant were infested by pouring an aqueous suspension of 100 H. microlobus individuals into a hole made in the sand at the base of the plant in the inner pot. Infested pots were maintained in a 75° F greenhouse for 3 months. Water was added only to the outer pot in an attempt to prevent leaching of nematodes from the inner to the outer pot.

At the conclusion of the experiment, nematodes were recovered from the inner pots by washing the sand 10 times, pouring the suspension through a Number 270 sieve 10 times, and carefully backwashing material on the sieve into a Petri dish. Roots of test plants were incubated in distilled water at room temperature for 48 hours, after which the water was sieved as above. H. microlobus specimens recovered from the sand and roots were counted.

Twelve pots were set up as before except that no plants were placed in the inner pots. Sand and soil from these pots were checked for H. microlobus and the number recovered was recorded.

A plant was considered a host for H. microlobus if more than 100 specimens were recovered 3 months after the plants were inoculated. If fewer than 100 were recovered from the inner pot, the soil in the outer pot was also processed. If results indicated that the test plant was not a host, the plant was again tested in the greenhouse. If results were still negative, the process was repeated until four tests, each with two replicates, had been completed. If no increase in population was detected in these tests, the plant was considered a non-host.

RESULTS

When 100 H. microlobus individuals were placed in sand in the absence of higher plants, five specimens were recovered from 1 pot, no specimens from 3 pots, and an average of fewer

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[2]Assistant Professor, Department of Plant Pathology and Botany, University of Minnesota.

[3]Taylor, D. P. 1960. Biology and host-parasite relationships of spiral nematode, Helicotylenchus microlobus. Ph. D. Thesis, University of Minnesota.

Table 1. Ability of test plants to support populations of H. microlobus based on populations recovered 3 months after inoculation.

Test plant: Family Scientific name	Common name	Variety, Clone, Cross, or Accession number	Reaction[a]
Pinaceae			
Picea glauca	white spruce		-
Gramineae			
Zea mays	corn	Pioneer 349	+
		W22 x A73	+
Zea mays var. rugosa	sweet corn	Morning Sun 72	+
		Golden Beauty	+
		Sugar King	-
		Golden Hybrid	-
Avena sativa	oat	Ajax	+
		Andrew	+
		Bentland	+
		Bonda	+
		Garry	+
		Gopher	+
		Minhafer	+
		Minland	+
		Ransom	+
		Rodney	+
		Sauk	+
Hordeum vulgare	barley	Fox	+
		Kindred	+
		Montcalm	+
		Peatland	+
		Traill	+
		Vantage	+
Secale cereale	rye	Emerald	+
		Tetra Petkus	+
Triticum aestivum	wheat, common	Lee	+
		Selkirk	+
		Thatcher	+
Triticum durum	wheat, durum	Langdon	+
		Ramsey	+
		Towner	+
		Yuma	+
Agrostis alba	redtop	--	+
Dactylis glomerata	orchard grass	--	+
Bromus inermis	smooth bromegrass	Minn. Clone 92-2	+
		Saratoga #1	-
		Wisc. Synthetic B	-
Festuca elatior	tall fescue	--	+
Poa pratensis	bluegrass	Park	+
		Marion	+
		Newport	-
Phleum pratense	timothy	Northland	+
		"Commercial Blend"	+
Liliaceae			
Allium cepa	onion	Hybrid Globe Elite	+
Chenopodiaceae			
Beta vulgaris	common beet	Ruby Queen	-
	sugar beet	AM 3S	+
		U.S.D.A. Acc. #1178	+
		U.S.D.A. Acc. #2056	+
		U.S.D.A. Acc. #2057	+
Cruciferae			
Brassica oleracea var. capitata	cabbage	Copenhagen Market	-
Brassica napobrassica	rutabaga	Laurentian	+
Brassica rapa	turnip	Purple-top-strap-leaved	+
Raphanus sativus	radish	Comet	+
Rosaceae			
Fragaria vesca	--	--	+
Fragaria chiloensis var. ananassa	strawberry	Robinson	-
		Arrowhead	-
		Gem	-
Fragaria virginiana	wild strawberry	--	+

[a]Reaction is based on whether a population increase occurred between the time of inoculation and the close of the experiment. Test plants that supported a population increase are marked "+"; whereas those that did not are marked "-".

Table 1 continued.

Test plant: Family Scientific name	Common name	Variety, Clone, Cross, or Accession number	Reaction[a]
Leguminosae			
Pisum sativum	pea	Perfected Wales	-
		Green Giant #65	-
Lupinus sp.	lupine	--	-
Lotus corniculatus	birds-foot trefoil	Empire	-
		Viking	-
Phaseolus vulgaris var. humilis	bean	Faribo Brittle Wax	+
		Improved Tender Green	+
		Greencrop	+
		Logan	+
		Pearlgreen	+
Glycine max	soybean	Acme	+
		Blackhawk	+
		Capital	+
		Chippewa	+
		Comet	+
		Earlyana	+
		Flambeau	+
		Grant	+
		Harosoy	+
		Hawkeye	-
		Monroe	+
		Norchief	+
		Ottawa Mandarin	+
Trifolium hybridum	alsike clover	"Swedish Tetraploid"	+
Trifolium pratense	red clover	Dollan	+
		Midland	+
		Wegener	+
		FC 32619	+
Medicago sativa	alfalfa	Grimm	-
		Lahontan	-
		Vernal	-
Melilotus alba var. annua	sweet clover	Hubam	+
Linaceae			
Linum usitatissimum	flax	B-5128	-
		Bison	-
		Norland	-
		Punjab	-
		Raja	-
Malvaceae			
Gossypium hirsutum	cotton	--	+
Umbelliferae			
Daucus carota var. sativa	carrot	Gold Pak	+
		Nantes	-
		Touchon	-
Solanaceae			
Solanum tuberosum	potato	Cobbler	+
		Red Pontiac	+
Lycopersicon esculentum var. commune	tomato	Big Boy Hybrid	+
		Big Early Hybrid	+
		Fireball	+
		Hybrid Giant King	+
		John Baer	+
		Marglobe	+
		Polaris	+
		Urbana	+
Capsicum frutescens var. grossum	sweet pepper	Acanqua	-
		Illinois F-5	-
		Keystone Resistant Giant	-
		Yolo Wonder A	-
Nicotiana tabacum	tobacco	--	+
Petunia hybrida	petunia	Celestial Rose	+
Cucurbitaceae			
Cucurbita maxima	squash	Blue Hubbard	+
Citrullus vulgaris	watermelon	Faribo 5-11	+
Cucumis sativus	cucumber	Smoothie	+
Cucumis melo	muskmelon	Pennsweet	+
Compositae			
Lactuca sativa	lettuce	Grand Rapids	-
Helianthus annuus	sunflower	Russian Mammoth	+
Zinnia elegans	zinnia	Pride of Dieldrin	-

than two specimens recovered from the 12 experimental pots. Without exception, all specimens recovered from these pots were very clear in appearance and moved only sluggishly.

One hundred twenty-seven varieties of plants, representing 48 botanical species and varieties contained in 14 families, were tested. Results are given in Table 1. Of all varieties tested, 94, or 74%, were rated as hosts for this nematode since more nematodes were recovered than were originally introduced. All varieties of the following major crops were rated as hosts: field corn, oats, barley, rye, common and durum wheat, sugar beet, red clover, soybean (except the variety Hawkeye), and potato. Non-hosts included pea, flax, and alfalfa.

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HOJA BLANCA DISEASE OF RICE FOUND IN MEXICO[1]

Peter R. Jennings and H. M. Beachell[2]

In early May 1960, hoja blanca, a virus disease of rice and certain grasses, was found in the State of Veracruz, Mexico. Typical symptoms of the disease were found on Echinochloa colonum and Digitaria sp. in a rice nursery infested with these grasses at the Cotaxtla Experiment Station. No diseased rice plants were observed.

Moderate to heavy infection was observed on E. colonum in seven commercial rice fields examined in the vicinity of Piedras Negras, Veracruz. One diseased rice plant of the native variety Jojutla was found.

Planthopper collections from rice and grasses at the Cotaxtla station yielded 1 specimen of Sogata orizicola Muir, 62 of S. cubana (Crawf.) and 113 of S. furcifera (Horvath). Collections of insects from rice and grasses in commercial rice fields near Piedras Negras included 2 specimens of S. orizicola, 5 of S. cubana and 19 of S. furcifera[3].

The finding of only one diseased rice plant is related to the low numbers of S. orizicola since this species is the only known vector of the hoja blanca virus in rice (1). The large amount of hoja blanca in grasses was correlated with the large numbers of S. cubana, which transmits the virus to grasses (2). Neither S. orizicola nor S. cubana is capable of transmitting the virus from grass to rice. S. furcifera has not been tested for transmission of the virus in Colombia because it is rarely encountered. However, it may be involved in infection of grasses.

The Veracruz area of Mexico has been carefully surveyed for hoja blanca since 1957. The 1960 observations constitute the first positive report of the disease in Mexico. These findings, combined with the recent outbreaks of hoja blanca in Guatemala and El Salvador, suggest that the disease may become of increasing importance in the Veracruz rice zones.

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COLOMBIAN AGRICULTURAL PROGRAM OF THE ROCKEFELLER FOUNDATION; CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE; THE TEXAS AGRICULTURAL EXPERIMENT STATION; AND THE TEXAS RICE IMPROVEMENT ASSOCIATION

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[1]Paper No. 131 of the Agricultural Journal Series of the Rockefeller Foundation.

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[3]Gratitude is expressed to Sra. Isabel Sanabria de Arevalo for the insect identifications.

SEVERAL UNIQUE STRAINS OF THE BARLEY STRIPE MOSAIC VIRUS

H. H. McKinney and Lester W. Greeley[1]

Chlorotic striping and mosaic are the usual reactions induced by the barley stripe mosaic virus (BSMV) in most commercial varieties of barley and wheat that have been observed. However, other reactions have been reported (3, 4, 5).

Dilution-transfer and selection studies of the virus have resulted in the isolation of strains[2] of the following six basic plant-reaction types, as determined with Atsel barley (C. I. 6250), Pilot wheat (C. I. 11428), Rescue wheat (C. I. 12435), Michigan Amber wheat (C.I. 11770), or Statesville oats (C. I. 4230): 1) latent in barley and wheat; 2) stripe mosaics in barley and wheat, and in some cases in oats, their virulence ranging from very mild to the "yellow-leaf" and the "white-leaf" types in barley and wheat; 3) coarse-blotch in barley and wheat; 4) fleck-blotch in barley and wheat; 5) spindle-stripe in oats, but white-leaf in barley and wheat; and 6) lethal-rosette in barley, wheat, and oats. Attempts to segregate a lethal factor, a rosetting factor, and some other species of virus from the lethal-rosette culture have failed thus far. A lethal "strain" and an albino "strain" appeared in some of our cultures, but attempts to maintain them in pure culture were not successful.

The coarse-blotch and the lethal-rosette strains are the only ones that have failed to pass to the next generation of barley seedlings through seeds produced by the infected plants. In five separate inoculation trials with the coarse-blotch strain, 553 viable seeds from 25 infected Atsel barley plants, 544 viable seeds from 8 infected Moore barley plants, and 1084 viable seeds from 35 infected Pilot wheat plants failed to produce any infected plants. The lethal-rosette strain permitted limited seed production in three inoculation tests with Manchuria barley, a tolerant variety. In preliminary tests involving 429 viable seeds from 22 infected Manchuria plants, no infection could be detected in any of the seedlings.

Table 1. Strains of the barley stripe-mosaic virus which were tested against the type-strain antiserum (1).[a]

Strain	Dehydrated tissue-sample number	Nature of viruliferous wheat- or barley-leaf tissue used: Dehydrated[b] Years stored	Dehydrated[b] Serological reaction	Fresh Serological reaction
Latent (from Pilot wheat seed)	808	2	positive	positive
Very mild stripe-mosaic, Ex-M 1B (from the latent culture)	---	--	not tested	positive
Mild stripe-mosaic (from Glacier barley seed)	799	2 1/6	positive	not tested
Stripe-mosaic, Ex-M 1 (resembles the type, - from the latent culture)	---	--	not tested	positive
Stripe-mosaic (type, tobacco passage)	854	1/6	positive	positive
Stripe-mosaic (type, Chenopodium album passage)	674	5 2/3	positive	not tested
Coarse-blotch (from the latent culture)	833	1 1/3	positive	positive
Fleck-blotch	836	1 1/4	positive	positive
Yellow-leaf	709	4 1/12	positive	positive
White-leaf	841	1	positive	positive
Mild oat strain	679	5 7/12	positive	not tested
Severe oat strain	592	7	positive	not tested
Spindle-stripe oat strain	---	--	not tested	positive
Lethal-rosette oat strain	---	--	not tested	positive

[a]Serological tests conducted by Scott (6).

[b]Methods of dehydration and storage were previously described (2).

[1]Collaborator and Agricultural Aid, respectively.

The authors wish to acknowledge the assistance of Henry J. Breen in connection with the testing of seed, carrying out inoculations, making observations, and recording notes.

[2]The term "strain" signifies an isolate, the characteristics of which remain essentially constant in series transfers.

The coarse-blotch strain and several stripe-mosaic strains were derived from the latent culture under circumstances which indicate that they evolved through mutation and possibly recombination in the latent culture after it had been isolated from a Pilot wheat seedling that became infected naturally through the seed from an infected field-grown plant.

The latent strain, when established in the test plant, has in all cases blocked or protected against reinfection by each of the strains listed in Table 1 and other isolates regarded as being BSMV, when used to challenge the latent strain in reinoculation tests. The results of these tests, and of serological tests conducted by Howard A. Scott (Table 1), indicate that these extremely divergent isolates, with reference to symptom induction and host adaptation, represent closely related strains of a species. Details of the serological studies conducted by Scott are reported elsewhere (6).

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ENDOTHIA PARASITICA ASSOCIATED WITH A CANKER OF LIVE OAK

Curtis May[1] and Ross W. Davidson[2]

In May 1960, Alden Eaton, Director of Landscape Construction and Maintenance, Colonial Williamsburg, Virginia, forwarded to us specimens of cankered branches of live oak (Quercus virginiana). Buff-colored mycelial fans suggestive of Endothia parasitica (Murr.) P. J. & H. W. Anders. were observed in and under the bark. Perithecial stromata and pycnidia present on bark of cankered area were typical of those of the chestnut blight fungus. Sizes of pycnidiospores and ascospores were also within the range of those of the chestnut fungus; pycnidiospores were one-celled, rod shaped, 3-4 x 1-1.5μ and ascospores two-celled, 7-9 x 3-4μ. Tissue-culture isolations from the cankers produced on malt agar colonies with the typical orange-colored mycelium of the chestnut blight fungus.

In culture the growth rate, color, and colony characteristics of the fungus isolated from the cankers were typical of Endothia parasitica rather than of Endothia gyrosa (Schw.) Fr. with which it was compared. Small scattered pycnidia with pycnidiospore masses were developing on and around the inoculum area of Petri-dish cultures in 7 days, at room temperature (25° C).

Within 3 weeks American chestnut[3] (Castanea dentata) inoculated with the fungus isolated from live oak developed rapidly expanding cankers typical of chestnut blight. The fungus was isolated from the cankers on the inoculated chestnuts.

Studies of the pathogenicity to live oak of the isolate from live oak are in progress.

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A NEW HOST OF PRATYLENCHUS COFFEAE FOR THE UNITED STATES

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Pratylenchus coffeae (Zimmerman, 1898) Goodey, 1951 has been recovered from dahlia tubers on two recent occasions by the Bureau of Plant Pathology, California Department of Agriculture.

The first instance resulted from a routine examination by the Los Angeles County Agricultural Commissioner of a single tuber in a small postal shipment from a bulb retailer in Michigan. The tuber, which showed no external symptoms of nematode injury, was incubated 8 days in a glass jar with a small amount of water. The nematodes were submitted to the Bureau laboratory in Sacramento for identification.

In the second instance, several dahlia tubers showing surface cracks and blemishes were sent to the Bureau laboratory by a San Mateo County agricultural inspector from a nursery in the Palo Alto area. The nematodes were extracted from the tuber tissue by a combination of Osterizer and Baermann funnel techniques. Additional samples from the same nursery have revealed the presence of P. coffeae in soil surrounding growing plants of several different varieties of dahlia. During the examination of one dahlia tuber from this property an estimated 50,000 P. coffeae were found.

Other hosts of P. coffeae published in the literature or listed in the records of the Plant Nematology Section, Crops Research Division, United States Department of Agriculture, Beltsville, Maryland, and of the California Department of Agriculture include: Aster sp., aster; Camellia japonica, camellia; Coffea arabica, coffee; Fragaria chiloensis, strawberry; Gossypium hirsutum, cotton; Malus sylvestris, apple; Medicago sativa, alfalfa; Musa paradisiaca var. sapientum, banana; Musa textilis, abaca; Musa sp., plantain; Solanum tuberosum, potato; Sweitenia mahogani, mahogany; Tagetes sp., marigold; Thea sinensis, tea; Trifolium pratense, red clover; and Tripsacum laxum, Guatemala grass.

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